

# **STUDIES ON INDIAN CICHLIDS**

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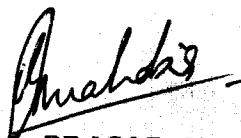
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### Declaration

I hereby declare that this thesis entitled "**STUDIES ON INDIAN CICHLIDS**" has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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**Certificate**

This is to certify that the thesis entitled "**STUDIES ON INDIAN CICHLIDS**" is the bonafide record of the work carried out by **Mr. GOPAL PRASAD MAHOBIA** under my guidance and supervision and that no part thereof has been presented for the award of any other Degree.

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## STUDIES ON INDIAN CICHLIDS

### LIST OF CONTENTS

PAGE No.

I.	PREFACE	
II.	ACKNOWLEDGEMENTS	
1.	GENERAL INTRODUCTION	
1.1.	Review of previous literature	1
1.2.	Scope of present study	8
2.	REVIEW ON THE PRESENT STATUS OF TAXONOMY OF INDIAN CICHLIDS	10
3.	COLLECTION OF FISH SPECIMENS FOR DIFFERENT STUDIES	17
4.	MORPHOMETRIC AND MERISTIC STUDIES	
4.1.	Introduction	22
4.2.	Methods	25
4.3.	Results of studies on Morphometric characters of <u>Etilapia suratensis</u>	27
4.4.	Results of studies on Meristic characters of <u>Etilapia suratensis</u>	46
4.5.	Results of studies on Morphometric characters of <u>Etilapia maculatus</u>	51
4.6.	Results of studies on Meristic characters of <u>Etilapia maculatus</u>	67
4.7.	Discussion	69
5.	BIOCHEMICAL STUDIES	
5.1.	Introduction	73
5.2.	Methods	82
5.3.	Results of Biochemical studies on <u>Etilapia suratensis</u>	88
5.4.	Results of Biochemical studies on <u>Etilapia maculatus</u>	101
5.5.	Discussion	107



<b>6.</b>	<b>BIOCHEMICAL GENETIC STUDIES ON <u>ETROPLUS</u> <u>SURATENSIS</u> AND <u>ETROPLUS</u> <u>MACULATUS</u>.</b>	
6.1.	Introduction	124
6.2.	Methods	133
6.3.	Results of Biochemical Genetic studies	147
6.3.1.	Protein expression in tissues	147
6.3.2.	Protein pattern variation of muscle tissue between different geographical areas	156
6.3.3.	Enzyme expression in tissues	158
6.3.4.	Genetic variation	162
6.4.	Discussion	167
<b>7.</b>	<b>CYTOTAXONOMIC STUDIES</b>	
7.1.	Introduction	192
7.2.	Methods	193
7.3.	Results and Discussion	197
<b>8.</b>	<b>INDUCED BREEDING AND REPRODUCTIVE POTENTIALS</b>	
8.1.	Introduction	200
8.2.	Methods	202
8.3.	Results and Discussion	204
<b>9.</b>	<b>SUMMARY</b>	208
<b>10.</b>	<b>REFERENCES</b>	i-xxvi

<b>6.</b>	<b>BIOCHEMICAL GENETIC STUDIES ON <u>ETROPLUS</u> <u>SURATENSIS</u> AND <u>ETROPLUS MACULATUS</u>.</b>	
6.1.	Introduction	124
6.2.	Methods	135
6.3.	Results of Biochemical Genetic studies	147
6.3.1.	Protein expression in tissues	147
6.3.2.	Protein pattern variation of muscle tissue between different geographical areas	156
6.3.3.	Enzyme expression in tissues	158
6.3.4.	Genetic variation	162
6.4.	Discussion	167
<b>7.</b>	<b>CYTOTAXONOMIC STUDIES</b>	
7.1.	Introduction	192
7.2.	Methods	193
7.3.	Results and Discussion	197
<b>8.</b>	<b>INDUCED BREEDING AND REPRODUCTIVE POTENTIALS</b>	
8.1.	Introduction	200
8.2.	Methods	202
8.3.	Results and Discussion	204
<b>9.</b>	<b>SUMMARY</b>	208
<b>10.</b>	<b>REFERENCES</b>	i-xxvi

## I. PREFACE

The population explosion and the critical food supply from the terrestrial environment have diverted man's attention towards aquatic environment to supplement his food requirements. Thus aquaculture is gaining importance for achieving additional production of proteinous food. In this context, the selection of productive strains of fish, crustaceans and molluscs for successful culture in different environmental conditions becomes a continuing process. Due to widespread degradation of natural aquatic environment often with decline or extinction of fish stocks, it has become important to evaluate their genetic diversity. The genetic resources of wild as well as farmed fish stocks can be improved and managed by understanding the genetic make-up and variability existing in them. Considerable progress has been achieved in the past on the evaluation of these aspects on various fishes, however, our knowledge on these in the Indian fishes is not adequate.

The endemic Indian cichlids represented by two species *Etroplus suratensis* (the pearl spot or green chromide) and *Etroplus maculatus* (the orange chromide) are traditionally considered to be suitable culturable species. They occur along the coastal tracts of peninsular India and some of the land locked freshwater lakes of the country. *Etroplus*

~~Suratania~~ the larger of the two is a delicious table fish considered to be more important for culture in brackish and freshwaters and considerable attention has been given in recent years to research and development programmes relating to the culture of this fish. Etroplus maculatus due to its smaller size is of lesser economic importance as a cultivable fish. It is however caught in the wild and also reared as an aquarium fish.

Present study has been undertaken to clarify the taxonomical status and variabilities of the species through study of morphometric and meristic characters as well as protein and isoenzyme variations. Variability in biochemical constituents of the tissues during the maturation cycle has also been studied. Attempts have been made to study the karyotypes and to induce the spawning under controlled conditions.

This thesis is the result of field and laboratory studies for a period of four years after completing the statutory course studies for one year in mariculture.

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## 1. GENERAL INTRODUCTION

### 1.1. Review of previous literature

The family cichlidae is comprised of over 700 species of fishes found in fresh as well as brackish water habitats in Africa, South America, North America and parts of Asia. The larger African lakes of Malawi, Victoria and Tanganyika contain about 500 endemic species of cichlids (Fryer and Iles, 1972). Their presence on the southern continents and islands such as Madagascar and Sri Lanka suggests that their ancestors were able to withstand either brackish or even marine waters.

Ectopoma is the only endemic Indian cichlid genus. Two well known species E. suratensis (Bloch) "the pearlspot" or "the green chromide" and E. maculatum (Bloch) "The orange chromide" occur along the coastal tracts of peninsular India and in some of the land locked freshwater lakes of the country.

E. suratensis is distributed widely in brackish and freshwater environments from Goa on the west coast to Chilka on the east coast. The pearl spot has been introduced in the different interior water sheds like dams and natural fresh water lakes from very early times. The erstwhile Madras Fisheries Department transplanted the species into

the interior districts of Bellary and Anantpur and in the farms at Sunkesula (Kurnool) and Ippur (Nellore), where it has established itself.

Fingerlings of this fish have been transplanted successfully from north Canara to the Mahim creek in Bombay (Kulkarni, 1947) in 1941 and in subsequent years from Sunkesula to the irrigation tanks of Baroda (Mosses, 1942 and 1944).

Fry from Madras have been introduced into the Bidyadhari area in Bengal (Jaganatham, 1946) and these bred successfully (Job and Chacko, 1947). The fish was introduced in the Hyderabad state in 1942 from Madras and has established itself (Rahimullah, 1946).

The orange chromide E. maculatus has more or less similar distribution and habitat as the pearlspot, but is of smaller size and hence is of lesser economic importance. It is reared as an aquarium fish also and for this purpose has been introduced into several countries outside India.

The orange chromide has been introduced by the erst-while Madras Fisheries Department into the Sunkesula farm and from there transplanted by the then Bombay Fisheries Department, where it figured regularly in catches (Kulkarni, 1947).

Though several early workers dealt with food, feeding, reproductive behaviour, biology, ecology and culture aspects



of these fishes, much more remains to be known about the taxonomy and details of their physiology in Indian waters.

Food and feeding of Etropius spp. mainly of E. suratensis were studied by Raj (1916) and Sebastian (1942). Bhaskara (1946) and Job et al., (1947) discussed briefly the biology of E. suratensis. Alikunhi (1952, 1957) dealt with the food and feeding habits of early fry, juveniles and adults of E. suratensis. Jhingaran and Natarajan (1969) studied the food and feeding habits of E. suratensis from Chilka lake. Gopalakrishnan (1970) mentioned in brief the food and feeding habits of E. suratensis. Prasadam (1971) reported on the food and feeding habits of E. suratensis from Pulikat lake. A comparative study of food and juveniles of E. suratensis collected from estuarine (Udyavara estuary) and fresh water (Yemmakara) environments of Mangalore were made by Devaraj et al., (1973). The anatomy and histology of alimentary canal of E. suratensis were studied by Thomas (1975). Varghese (1976), while studying the biology, morphology and development of cichlids of Kerala made observations on the food and feeding habits of E. suratensis and E. maculatus collected from Cochin.

Sumitra (1978) and Krishnakumari et al., (1979) studied the food conversion efficiency in E. suratensis. Jayaprakash et al., (1979) studied the food and feeding habits of Etropius maculatus.

Caloric values of ingested food of E. suratensis grown in a culture pond was studied by Sumitra et al., (1981). Jayaprakash (1980) studied the food and feeding habits of different size groups of E. suratensis collected from Veli lake Trivandrum. Growth and food conversion efficiency in E. suratensis in relation to different feeding levels were studied by Sumitra et al., (1982). Digestibility of an aquatic macrophyte by cichlid E. suratensis (Bloch) with observations on the relative merits of three indigenous components as markers and daily changes in protein digestibility was studied by De silva and Ferera (1983). De Silva et al., (1984) studied the food and feeding habits of E. suratensis in Sri Lanka.

Various authors have given brief informations on breeding season and habits of E. suratensis and E. maculatus. Mention may be made of the contributions of Jerdon (1848), Thomas (1870), Raj (1916), Panikkar (1920, 24), Jones (1937, 46) and Rice (1944). Raj (1916) reported April and May as breeding season for E. suratensis in Madras and in Travancore reported to breed twice in a year, in May to June and again from November to February with peak in January. Jones (1937a) reported July and August as breeding season for E. suratensis in Adyar river, Madras and May and June in Travancore. He mentioned that E. maculatus breeds during August and September. In Chilka lake E. suratensis was reported to breed throughout the year with peak in December to February and April to May

(Alibekhi, 1957), Varghese (1976) reported that E. maculatus breeds during July-August (North-West monsoon period) in Cochin backwaters. Oogenesis in E. suratensis was studied in detail by Jayaprakash and Balakrishnan (1981). They report June and July & November to March as breeding periods for E. suratensis in Veli lake, Trivandrum.

Jayaprakash et al., (1979) studied the breeding biology of E. maculatus. They reported two spawning seasons in this species, one in June-July and another in November-December and January in the same area. Ward and Samarakoon (1981) stated that E. maculatus breeds during February and July, while E. suratensis breeds twice during the pre-monsoon season, during January to April and again in July, in Sri Lanka.

Ward and Barlow (1967) studied the maturation and regulation of glancing off the parents by E. maculatus and effect of size on courtship was studied by Barlow (1968). Quarterman and Ward (1969) studied the development and significance of two motor patterns used in contacting parents by young E. maculatus. Cole and Ward (1969) studied the communicative function of pelvic fin flickering in E. maculatus and described an analysis of parental recognition of the young E. maculatus. Barlow (1970) studied a test of appealment and arousal hypothesis of courtship in E. maculatus. A cleaning symbiosis between E. suratensis and E. maculatus

was reported by Wymen and Ward (1972), digging behaviour was studied by Kuso (1974) in detail. Increased aggressive activity between male and female in isolated pairs of E. maculatus by the drive hypothesis was studied by Reyer (1975). Territorial and reproductive behaviours in E. suratensis and E. maculatus in Negombo Lagoon of Sri Lanka, were studied by Ward et al., (1976 a and b) and Wymen and Ward (1973). Ecological and behavioural interactions of E. suratensis and E. maculatus were observed by Ward and Wymen (1977). Rechten (1980 a and b) gave a note on the reproductive colouration of E. maculatus and also studied its brood relief behaviour in detail.

Ward and Samarakoon (1981) studied the reproductive tactics of the genus Etroplus in Sri Lanka while Lamon and Ward (1983) studied the measurements of reproductive efforts from successive reproductive cycles for E. maculatus. Parental egg care behaviour and fanning activity was observed by Joran and Ward (1983) in E. maculatus of Sri Lanka.

Embryology and larval development of Etroplus spp. were studied by Raj (1916), Panikkar (1920), Jones (1937a), Alikunhi (1952) and Varghese (1976).

Karyotype studies were made on E. suratensis and E. maculatus by Natarajan and Subramanyam (1974) and on E. suratensis by Rishi and Singh (1982).

Studies on ecological and physiological aspects of Etroplus were made by Job et al. (1947). Pannapathi Rao (1958) reported the salinity tolerance of E. maculatus. Parvatheswara Rao (1959) studied the Oxygen consumption in E. maculatus in relation to size and temperature. Structural changes in the gills, intestine and kidney of E. maculatus adapted to different salinities were reported by Virabhadrachari et al., (1961) and adaptive physiology in E. maculatus was studied by Pannapathy Rao (1962). Influence of different temperature-salinity combination on the Oxygen consumption in E. maculatus was studied by Parvatheswara Rao (1965). Visual pigments in E. maculatus were reported by Virabhadrachari et al., (1967). Parvatheswararao (1967) studied about some mechanisms underlying thermal and salinity acclimation in E. maculatus. Carbonic anhydrase activity as a function of salinity acclimation was studied by Krishnamurthy (1969). Possible role of thyroid in the thermal acclimation, compensatory metabolic regulation to seasonal thermal stress, metabolic compensation during thermal acclimation in the tissues and size and thermal dependence in the metabolism of tissues in E. maculatus were studied in detail by Parvatheswara Rao (1970, 1971, 1972 and 1977). Parvatheswararao (1975) and Ramakrishna (1977) studied about the visual pigments in E. suratensis. Hoffman (1978) investigated the effect of a quaternary ammonium compound lethal limits under different conditions, the physiology ( $O_2$  consumption, food conversion etc.) growth of

the treated animals and their successors and discussed the changes of epidermal structures. Vijaya lakshmi (1980) studied the effect of LD 50 exposure of E. maculatus to Sumithion (Penitrothiden) on tissues respiration and enzyme activity in E. maculatus.

Jayaprakash (1980) gave an account of the present status and possibilities of aquaculture of E. suratensis in Kerala. Sumitra et al., (1981) reported aquaculture production of E. suratensis to be 437.5 kg/ha/yr in Ele-Dhauji fish farm, Goa.

Production of E. suratensis in monoculture was reported to be 631 and 800 kg/ha/yr, and in mixed culture along with mullets and Chanos 543 kg/130 days by Gopinath (1981) and Mathew (1981) reported a net production of 836 kg/6 months and 1242 kg/7½ months, when E. suratensis, Chanos chanos and Mugil species were cultured together in Vytilla centre of Kerala Agriculture University.

## 1.2. Scope of present study

In view of wide-spread degradation of natural aquatic environments, often with decline or extinction of fish stocks, it has become important to evaluate the genetic diversity of fish resources. During the last decade considerable progress has been made towards understanding the genetic make-up and

variability of the wild as well as farmed fish stocks for the management and improvement of their genetic resources. However, our knowledge of these aspects in the Indian cichlids is scarce.

The study aims to clarify the taxonomic status of the species through study of morphometric and meristic variations and protein patterns variation of fishes from different geographical areas of peninsular India. Attempts have also been made to understand the genetic make-up of populations of both species in Cochin backwaters through isoenzyme study.

The biochemical parameters such as, moisture, protein, carbohydrate, lipid and ash were estimated in the blood, muscle, liver and gonad at each maturity stage.

Studies on chromosomes and induced breeding have also been made. Various dosages of hormones and steroids were tried to induce spawning in Ectopius suratensis in the laboratory conditions.

The present studies carried out on the morphological, biochemical and chemotaxonomical aspects on the two species of Indian cichlids E. suratensis and E. maculatus form original contribution enriching the present knowledge on cichlids. The results of this investigations it is hoped would help in the management and improvement of the aquaculture schemes of Indian cichlids.

## 2. REVIEW ON THE PRESENT STATUS OF TAXONOMY OF INDIAN CICHLIDS

As presently recognized the cichlidae is one of the most speciose and ecologically diverse family of percoid fishes. It is the second largest family in the Perciformes including over 700 species.

Fishes of the genus Ectopoma (Bloch) of the family cichlidae are euryhaline and endemic to peninsular India and Sri Lanka. Earlier workers like Day (1865, 1878, 1889) and Gunther (1862) included the genus under the family Chromidae. Diagnostic characteristics of the family chromidae have been given by Gunther (1862) as follows:-

### Family Chromidae

Chromidae, Muller, Berl. Abhandl - 1844

Body elevated, oblong or elongate, scaly, the scales generally being ctenoid. Lateral line interrupted or sub-interrupted, one dorsal fin, with a developed spinous portion, three or more anal spines, the soft anal similar to the soft dorsal. Ventral fins thoracic with one spine and five soft rays. Teeth in the jaws small; palate smooth. The lower pharyngeal bone triangular, with a median suture. Branchiostegals five or six, gills four, pseudobranchiae none; air bladder present. Pyloric appendages none (or in small numbers). Stomach with a caecal appendage. The abdominal and caudal vertebrae are nearly equal in number, or the number of the



former is slightly more.

Gunther (1962) included Etiopling under the family cichlidae. He gave an account of the family and stated that true cichlids are distinguished from true perches (Percidae, Centrarchidae, Nanidae) by the presence of only one nostril on each side of the head, which serves simultaneously as entrance and exit for the nasal cavity.

The body is usually deep to very deep, often even disc-shaped and strongly compressed, rarely elongate and low.

The almost invariably large head becomes further accentuated in the males of many species (both sexes in some) through the development with age, of swollen cushions of fatty tissue in the snout region and the hinder part.

The mouth is protrusible, usually broad and often bordered by swollen lips.

The long-based dorsal fin and the short-based anal fin always consist of anterior spinous and posterior soft rayed portions and their hinder ends, especially common in males, may be pointed or even considerably produced.

In some cichlids as in the genus Pterophyllus these fins may be extremely large and are spread out like sails, caudal fin is rounded, posteriorly or squarely truncated, more rarely emarginate.

The lateral line in cichlids is usually in two parts, the upper portion extends from the gill cover to below the soft dorsal, while the lower portion appears as though the hinder part of the upper lateral line has been broken off and transported to lower level.

Boulenger (1898) believed the cichlidae to be "very natural family of perciform Acanthopterygians" and he and subsequent authors were content to present familial diagnoses combining a series of characters which, when taken in combination distinguished the cichlids from other taxa (Regan, 1913; Bertin and Arambourg, 1958; Fryer and Iles, 1972; Chichoki, 1976). Of these characters some are plesiomorphic at the perciform level e.g. ventral fins thoracic, with one spinous and five soft rays, while others, such as a single nostril on either side of the head, interrupted lateral line and fifth ceratobranchials united in to a single element have a more restricted distribution. However, none of these characters is unique to "that entity that classify as the cichlidae" (Greenwood 1972).

Liem (1973) introduced a key to define the family cichlidae on the basis of a particular configuration of the cichlid pharyngeal jaw apparatus as follows:-

- The presence of two synovial basipharyngeal joints
- The sutural connection of the two-fifth ceratobranchials into a single lower pharyngeal element.

- A shift in the insertion of the paired fourth levator externus muscles from the fourth epibranchials to the "muscular processes" of the lower pharyngeal element.

Stiassny (1981) investigated four apomorphic characters to define the family cichlidae by the current methods of cladistic analysis.

- The loss of major structural association between parts  $A_2$  and  $M$  of the adductor mandibulae and the muscular insertion of a large ventral section of  $A_2$  on the posterior border of the ascending process of the angulo-articular.
- The presence of an extensive cartilaginous cap on the anterior margin of each second epibranchial bone.
- The presence of an expanded head of each fourth epibranchial bone.
- The presence of characteristically shaped and distributed micro-branchial spines on the gill arches.

The species of the genus Etoplus (Bloch)

The systematics of the genus Etoplus has been a confusing problem to many workers. Etoplus was formerly know as Chaetodon (Bloch, 1790, 1795; Bloch and Schneider, 1801; Lacepede, 1803, and Hamilton, 1822). Lacepede(1803) considered E. maculatus as Glyphisodon kakaitzel and

Swainson (1939) as Microgaster coruchi and E. suratensis as Chaetolabus suratensis. Cuvier and Valenciennes (1830) for the first time reported Etroplus under the synonymy of Chaetodon, which was followed by subsequent authors (Jerdon, 1848; Bleeker, 1853; Gunther, 1862; Day, 1865, 78, 89; Munro, 1955 and Gunther, 1962).

Commenting on the Etroplus described by Bleeker (1853), Gunther (1862) states as follows:-

"According to a communication from Dr. V. Bleeker, he intends to separate this species generally from E. maculatus retaining the name of Etroplus for the former, and adopting that of Pseudetroplus for the latter. He writes that the characters of the new genus are tricuspid teeth, and a scaly sheath along the base of the dorsal and anal fins. I find these characters equally developed in E. suratensis and E. maculatus and I can come to no other conclusion than that Dr. V. Bleeker either has a third species, different from both or that he has taken the characters for Etroplus from a very old specimen of E. suratensis, in which incisions in the front teeth have become obsolete".

Day (1869) reported three species of etroplus from Indian waters i.e., E. canarensis, E. maculatus and E. suratensis out of which E. canarensis is confined to north Canara region only and has not been reported by any other workers. However,

E. maculatus and E. suratensis are the two widely accepted species which are reported by various earlier and recent workers from Indian and Sri Lankan waters (Jerdon, 1848; Bleeker, 1863; Gunther 1862; Day 1865, 1878, 1889; Munro, 1955; Gunther, 1962).

Embryology and larval development of Etroplus spp. were studied by Raj (1916) Panikkar (1929); Jones (1937, 1946), Alikunhi (1952) and Varghese (1976). They reported that the larvae of both E. maculatus and E. suratensis are very much alike except for the slightly larger size of the latter. The difference in the nature of the chromatophores is so little that it is very difficult to distinguish one from the other.

According to Day (1878) the main characteristics of the two species of Etroplus are as follows:-

1. Etroplus suratensis

Fin Formula; B-vi,  $\frac{D-18-19}{14-15}$ , P-17 V-1/5  $\frac{A-12-13}{12-13}$

C-16. L1-35-40 Ltr.-54/17.

Teeth in a single row in each jaw, compressed and with a small lobe on each side, whilst posterior to them in both jaws are one or two rows very much smaller, but of the same description and separated by a short interspace from the outer row. Caudal fin slightly emarginate.

Light green colour, body with eight vertical bands, the first passes over the occiput, the last across the base of the caudal, and the other six are intermediate. Most of the scales above the lateral line have a central white pearly spot, while there are some irregular black spots over the abdomen. The dorsal, caudal, ventral and anal area of dark leaden colour, the pectoral yellowish with a jet black base, maximum size 31.0 cm.

## 2. Strobelius maculatus

Fin formulas: B-vi; D-~~17-20~~<sub>8-10</sub>; p-14; V-1/5; A-~~13-15~~<sub>8-9</sub>

C-16; Ll-35; Ltr.-16-19; Vert.15/13. Teeth trilobate and not quite touching one another, caudal lunated. Body colour is yellowish, with a greenish black and about seventeen horizontal lines of deep golden spots; occasionally there are a few spots along the dorsal fin, the back and the abdomen and also on the anal. Along the lateral. there are three black finger marks, the central being the largest and darkest, ventral and anal fins of deep black, maximum size 8 cm.

Both the species can be easily distinguished, especially by the structure of their caudal fins, spots of the body and the colour patterns.

### 3. COLLECTION OF FISH SPECIMENS FOR DIFFERENT STUDIES

For the present studies Etroplus suratensis specimens were collected from eight localities, four on the west coast, Cochin (backwaters), Mangalore (Mulky Fish Farm), Karwar (Sunkeri estuary), Goa (Zuery estuary) and three localities on the east coast - Pondicherry (Channambuara estuary), Madras (Muthukadu Fish Farm and Pulikat lake) and Hyderabad (Himayat Sagar fresh water lake).

Specimens of E. maculatus were collected from Cochin back waters, Muthukadu fish farm, and Pulikat lake, Madras and Himayat sagar lake, Hyderabad.

A total number of 508 specimens of E. suratensis and 267 specimens of E. maculatus were studied for morphometric and meristic characters. Specimens were collected from April, 1983 to September, 1983. They were preserved in 5% formalin and analysed within 15 days after collection.

For biochemical estimations specimens were collected from July, 1983 to March, 1984. Adults and juveniles of E. suratensis and E. maculatus were collected from the Panchalam Fish landing centre at Cochin. Live specimens were transported to the laboratory in water of the collection site in a 20 l. capacity plastic bucket. Sampling was done during morning hours between 6.30 to 8.00 hrs.

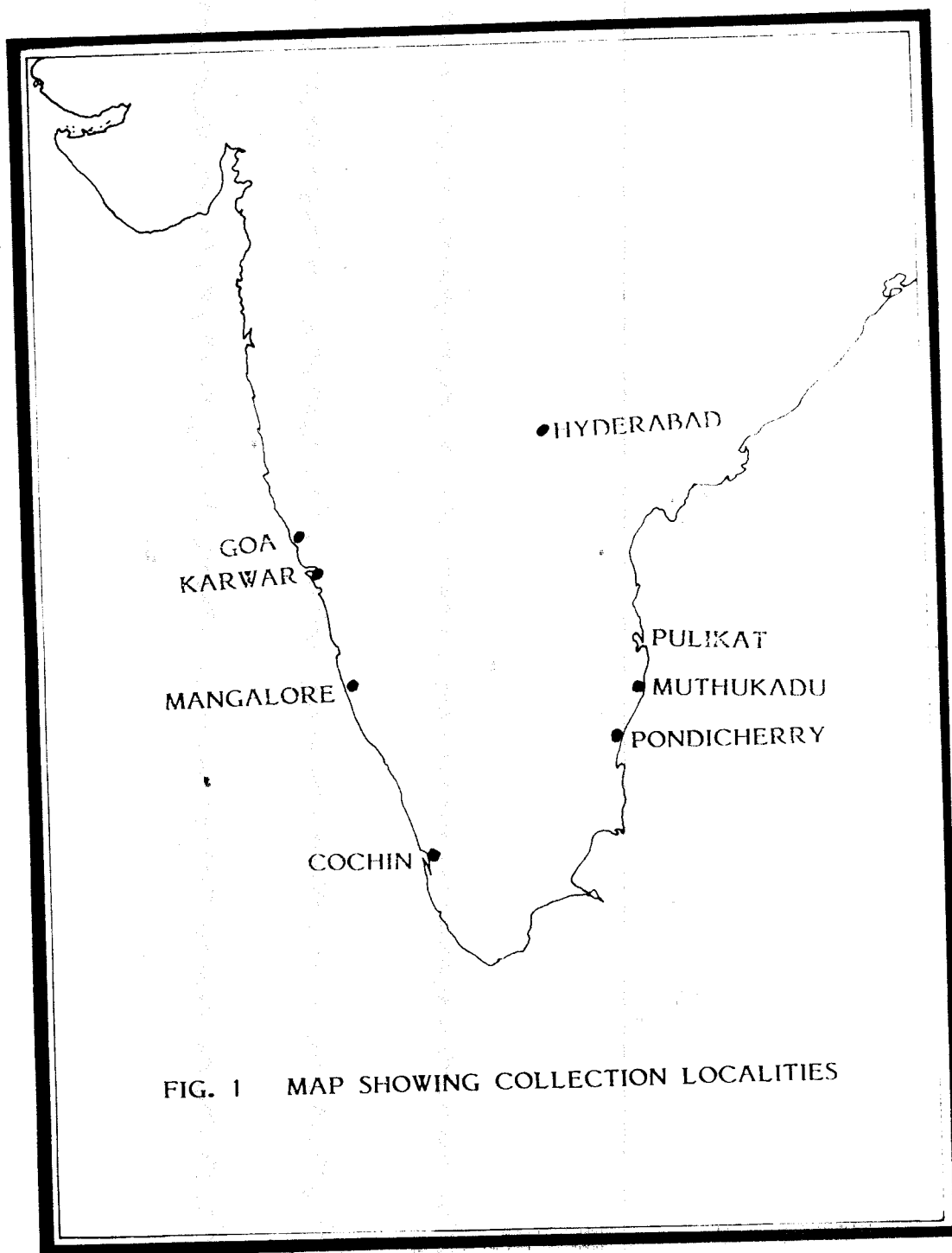


FIG. 1 MAP SHOWING COLLECTION LOCALITIES



Details of samples collected from different localities for morphometric and meristic studies.

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Species	Localities/Sample size							Total
	Cochin	Mangalore	Karwar	Goa	Pondicherry	Madras	Hyderabad	
<u>E. suratensis</u>	52	83	76	95	58	125	19	508
<u>E. maculatus</u>	83	..	..	..	..	124	60	267

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Major part of collection of fish sample for chaemotaxonomical studies were from the brackish waters around the Prawn Culture Laboratory at Narakkal and the Pachalam Fish Landing Centre at Cochin, Kerala. Some samples for the above studies were also collected from other localities, namely; Mulki Fish Farm, Mangalore; Sunkeri Estuary, Karwar; Zuari estuary, Goa; Chunnambuara estuary, Pondicherry; Muthukadu Fish Farm and Pulikat lake, Madras and Himeyat Sagar lake, Hyderabad. Samples collected at the outstation centres on the west coast were studied at NIO, Goa, CMFRI Research Centre, Karwar and Fisheries College Mangalore. Those from Madras and Pondicherry were studied at the Muthukadu field Laboratory of CMFRI. The Hyderabad samples were studied at CCRLB, Hyderabad.

Live specimens collected at Cochin were transported to the laboratory in 18 litre capacity plastic transportation bags filled two-thirds with oxygenated brackish water of the collection site. The live fish were transferred to perspex aquarium tanks (60 x 30 x 30 cms) filled three-fourths with water of the same salinity as at the collection site and were allowed to acclimatize. Sufficient aeration was provided with an air compressor. Water was changed and the faecal matter siphoned off once a day. Specimens showing distress were immediately subjected to analysis and active specimens were allowed to acclimatize. They were starved for 48 hours.

After 48 hours wet feed containing the following ingredients was given. The diet was accepted very well.

Diet ingredients	% (by wt.)
Fish meal	40
Rice bran	10
Ground nut oil cake	20
Tapioca powder	20
Mineral mixture	6.0
Vitamin Tablets	1 No./g. feed

At outstation centres, live specimens were transported to the respective laboratories in plastic buckets filled with water of the collection site.

Specimens from the fish markets or landing centres were transported in an ice box (36 x 20 x 23 cms) containing broken ice. These specimens were either analysed immediately or within 24 hours of collection; in the latter case, specimens were kept in a deep freeze at  $-4^{\circ}\text{C}$ .

For cytotaxonomical studies specimens were collected only from Cochin back waters. The collection method was same as described for the chaenotaxonomical studies.

For induced breeding and reproductive potential study, live fishes were collected with a cast net from brackish water canals of Narakkal during December, 1984 to March, 1985 period.

Live specimens were transported to the laboratory in a fibre glass tank (75 x 50 x 50 cms) filled two-thirds with the water of the collection site. Experiments were conducted in two sizes of plastic pools; diameter-height, 91-61 cms and 366-122 cms. Salinity of the water was maintained at 12‰ and temperature between 28-30°C throughout the experiments.

#### 4. MORPHOMETRIC AND MERISTIC STUDIES

##### 4.1. Introduction

The identification of discrete populations based on racial studies could lead to locating populations of culturable species like Etroplus with many desirable characters for culture purposes. Ahlstrom, (1957) states that under conditions of partial or complete isolation of groups of fish, slight difference in morphological or meristic characters will be preserved in each group. The small differences will not necessarily be apparent in individual specimens but often only in an average of a large number of specimens. The significance of differences is appraised by means of statistical procedures based on the theory of probability. The differences might be due to either environment or hereditary factors.

It has been shown by experimental work on several species of fishes that environmental factors such as temperature, salinity, pH and Oxygen tension can modify the expression of the genes responsible for meristic characters (Taning, 1952; Orska, 1957; Trojner, 1977; Dunham et al., 1979; Balon 1980; Todd et al., 1981 etc.). The number of serially repeated characters evidently alters with the environmental changes associated with latitude has been demonstrated by Hubbs, (1926); and Gross, (1977). Thus, the

phenotypic expression of characters represents a complex reciprocity between epigenetic, physiological and environmental factors.

Different workers have adopted different methods on various fishes for population studies. Some workers have taken into consideration only meristic counts, while others have studied both meristic counts as well as morphometric characters. In some cases, a number of characters analysed might not show significant differences, while in others, a single character might well be useful to denote distinct population.

Pillay (1957) differentiated the populations of Hilsha ilisha (Hamilton) of the river Hooghly from those of Chilka lake, based on morphometric and meristic characters. Similarly morphometric and meristic characters have been used to distinguish the populations of Chela labuca (Bass) from India, Burma, Malaya and Ceylon (Silas, 1958); of Anchoviella commersonii from Andhra Coast (Rao, 1965); Sardinia godavariensis from Godavari and Hooghly estuaries (Rao and Joglekar, 1967); of Goat fishes (family Mullidae) (Thomas, 1969) of saurida tumbil (Bloch) (Rao, 1982).

Population studies using morphometric and meristic characters were carried out in cichlid fishes Tilapia spilargus and T. zillii by Trewavas, (1966); T. aurea

and T. gilli by Chervinski (1967, 1968 a & b) Cichlasoma sieboldii and C. tuba by Bussing and Michael (1975) and in genus Nectropus by Rogers (1981).

Godsil (1948) in a preliminary population study of the yellow fin and albacore (Thunnus albus) from Japan and Hawaiian islands used only body proportions, since meristic counts were found unsatisfactory. Pilley (1951) and James (1967) also used only morphometric characters to study the population of Barbus and Lepidogobius intermedius respectively.

Meristic characters have been used by various authors on a number of fishes for population studies such as on Indian mackerel Scomber indicus (Balakrishna, 1965); Greenland halibut Reinhardtius hippoglossoides (Templeman, 1970); oil sardine (Ennigeri, 1978); beaked red fish Sebastes mentella and S. fasciatus (Ni, 1982).

The ratios between various body proportions differ at different stages of life history in fishes (Godsil, 1948; Marr, 1955). To overcome this difficulty, the comparison of different samples is based on the comparison of the regression of one dimension on that of another, taken as measurement of overall size. This method of regression has been used by Godsil (1948), Pilley (1957), Prasad (1958), Tandon (1962), Thomas (1969), James (1967).

The present study of morphometric and meristic characters has been taken up to clarify whether Etroplus suratensis and E. maculatus from Indian waters are comprised of one or more populations.

#### 4.2. Methods

##### a. Measurements and counts

Thirty one morphometric and nine <sup>meristic</sup> characters were selected for study. The method of measurements and the meristic counts were made on the lines described by Holden and Raitt (1974) and Baral et al., (1977).

##### Morphometric characters (Fig. 2-4)

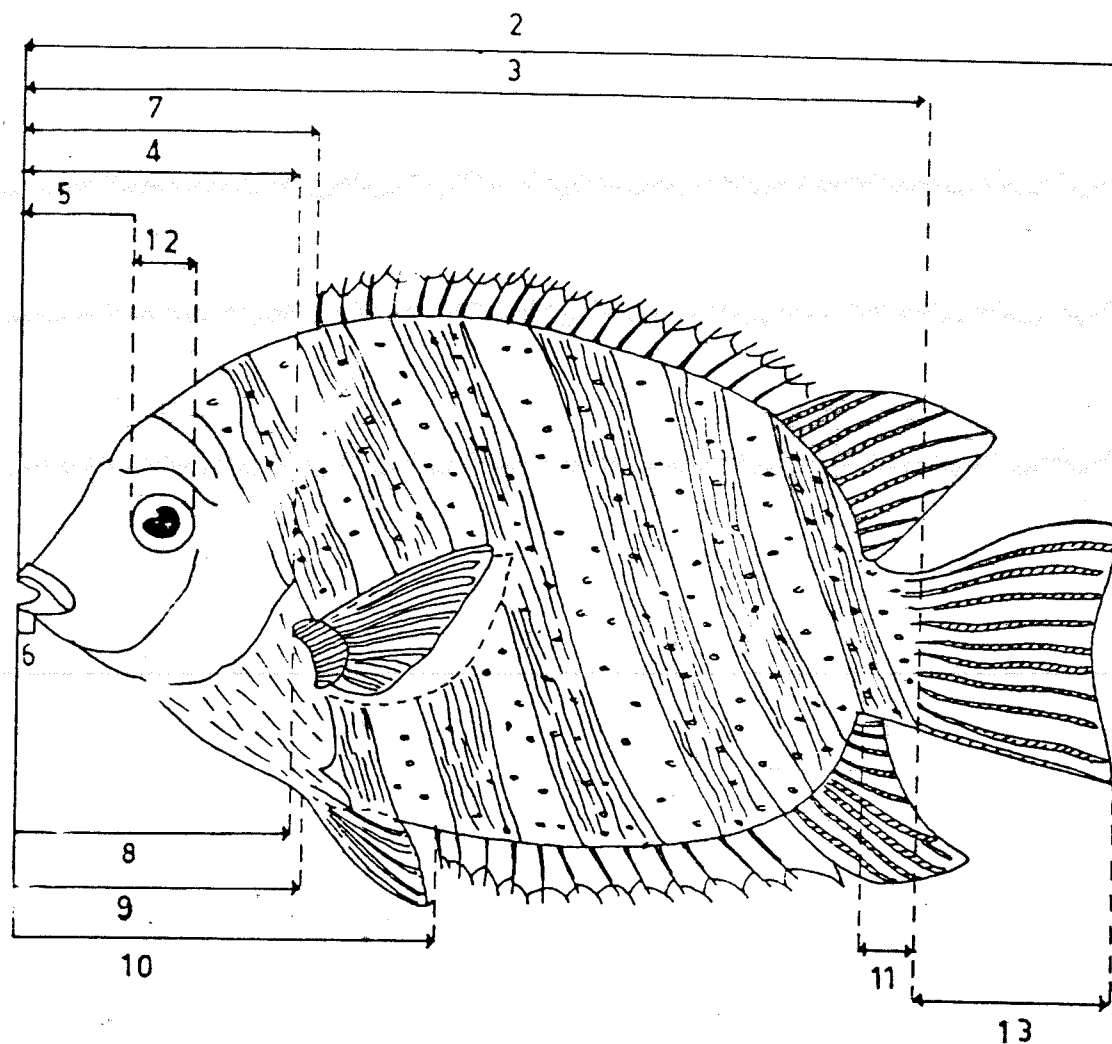
1. Weight (WT)
2. Total length (TL)
3. Standard length (SL)
4. Head Length (HL)
5. Snout length (SNL)
6. Lower jaw length (LJL)
7. Pre-dorsal distance (LD)
8. Pre-pectoral distance (LP)
9. Pre-ventral distance (LVL)
10. Pre-anal distance (LA)
11. Caudal peduncle length (LPL)
12. Eye diameter horizontal (EYL)



13. Caudal fin length (CFL)
14. Eye diameter vertical (EYD)
15. Head depth (HD)
16. Cheek depth (CHD)
17. Greatest depth (GH)
18. Dorsal-anal depth ( $D_1A_1$ )
19. Perpendicular anal depth ( $A_1A_2$ )
20. Caudal peduncle depth (CPD)
21. Snout width across pre-orbital process (POW)
22. Snout width across lachrymal (LAW)
23. Width across pectoral fin (PP)
24. Greatest width (GB)
25. Inter-orbital width (IOW)
26. Caudal peduncle width (CPB)
27. Lower jaw width (LJW)
28. Length of pectoral fin (PH)
29. Length of Ventral fin (VH)
30. Largest dorsal fin ray (LDFR)
31. Largest anal fin ray (LAFR)

#### Meristic characters

1. Dorsal fin branched (DFBr)
2. Dorsal fin spinous (DFSp)
3. Anal fin branched (AFBr)
4. Anal fin spinous (AFSp)
5. Vertebrae abdominal (VA)
6. Vertebrae caudal (VC)
7. Gill rakers upper (GRU)



**FIG.2: DIAGRAM OF *ETROPLUS SURATENSIS* SHOWING DIFFERENT MORPHOMETRIC LINEAR MEASUREMENTS.**

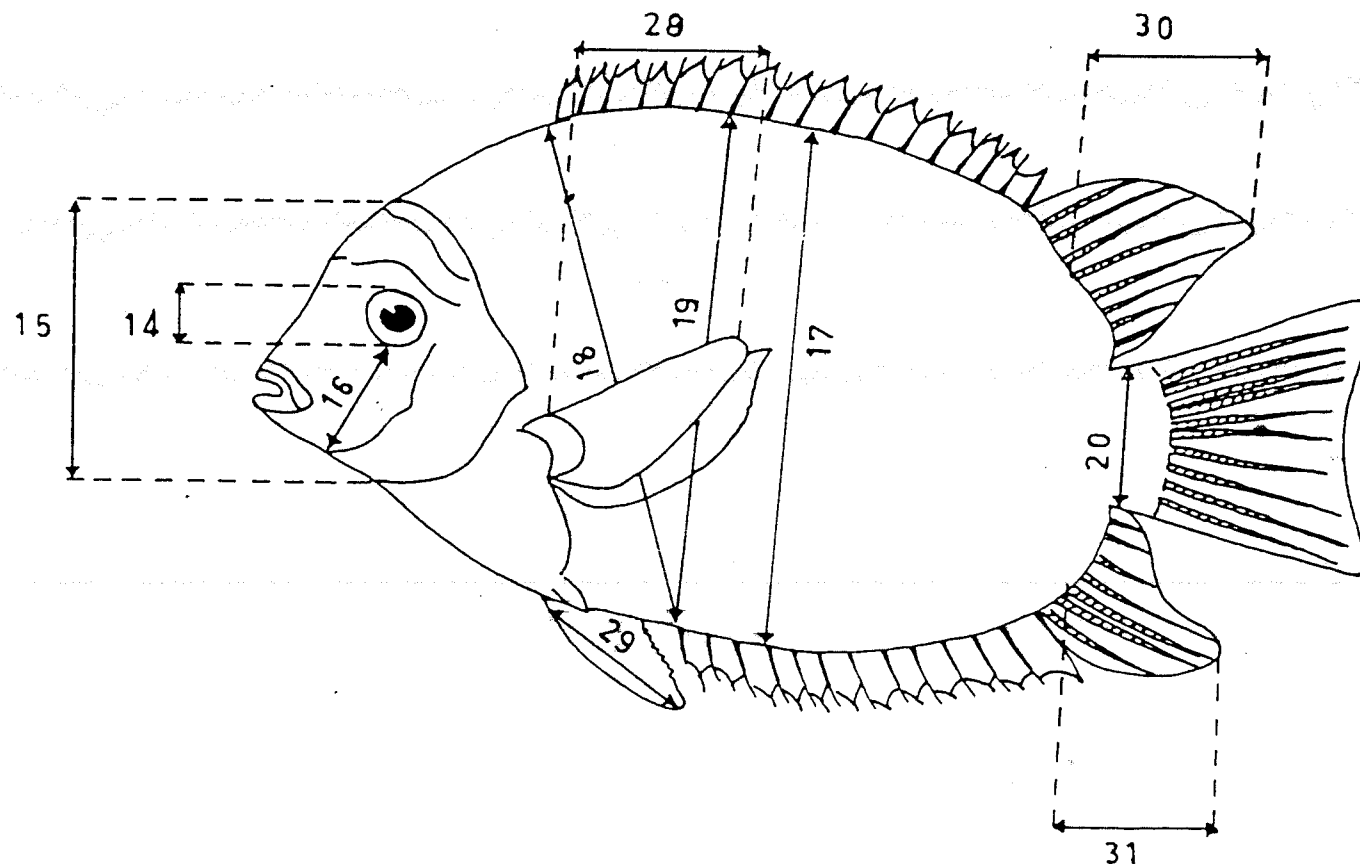


FIG.3: DIAGRAM OF *ETROPLUS SURATENSIS* SHOWING DIFFERENT MORPHOMETRIC VERTICAL MEASUREMENTS

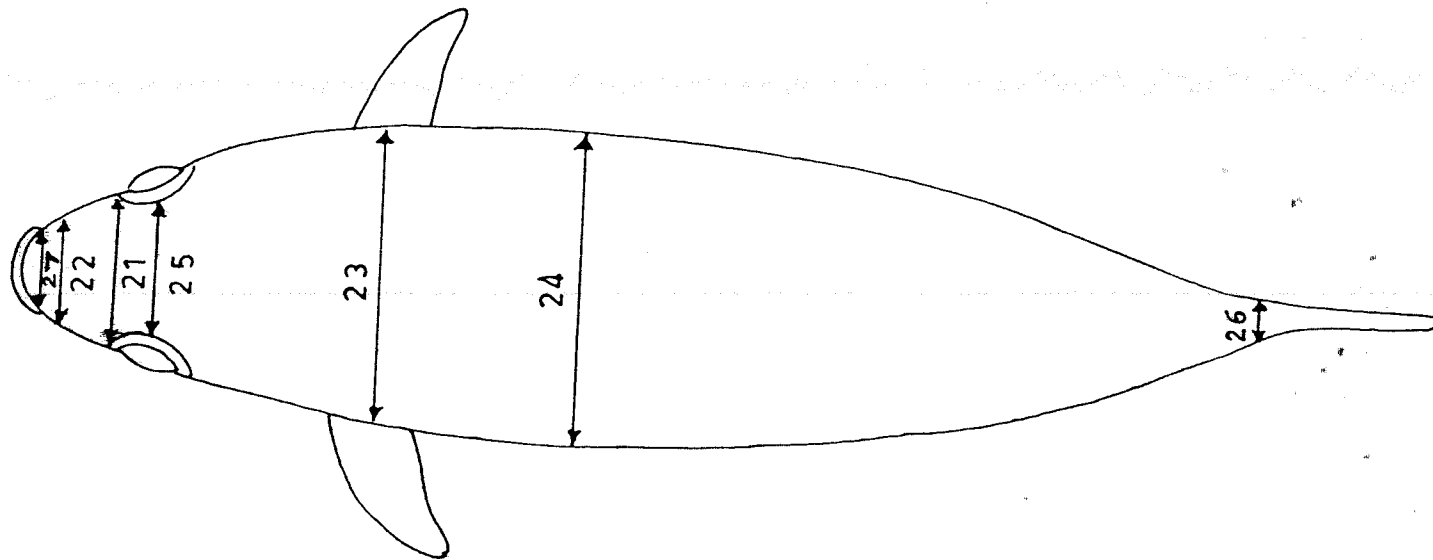


FIG. 4. DIAGRAM OF ETROPLUS SURATENSIS SHOWING DIFFERENT MORPHOMETRIC LATERAL MEASUREMENT.

7. Gill rakers upper (GRU)
8. Gill rakers lower (GRL)
9. Gill teeth (GT)

b. Statistical analysis

Morphometric data were computed for mean, standard deviation, correlation matrix, and variance - covariance matrix. Correlation values were observed to be highly significant in different localities for nine characters i.e., WT, SL, LIL, CFL, EYD, GH, PP, IO and LDPR. The analysis of dispersion (Rao, 1974) for these nine characters was carried out to see the variation in both species between different localities.

Mean and standard deviation of the different meristic counts were obtained. The standard error of mean has also been calculated by using the formula  $\frac{\sigma}{\sqrt{n}}$ , where 'n' is the number of specimens in the sample. By applying 't' - test no significance of mean values of different meristic characters were obtained between the samples of different localities.

4.3. Results of studies on Morphometric characters of Etroplus suratensis

Body weight (WT)

There was significant difference in body weight between sexes and in pooled out sexes in all localities (Table 1 & 2).

TABLE - 1 MEAN AND STANDARD DEVIATION OF DIFFERENT CHARACTERS BETWEEN SEXES OF  
ETROPLUS SURATENSIS IN DIFFERENT LOCALITIES.

Sl. No.	Character	Sex	Cochin.	Mangalore	Karwar	Goa	Pondicherry	Muthassur	Pulikat lake	Hyderabad
1	WT	Male	108.01 ( $\pm 41.95$ )	58.67 ( $\pm 53.22$ )	44.64 ( $\pm 27.44$ )	47.12 ( $\pm 23.38$ )	78.25 ( $\pm 26.97$ )	78.64 ( $\pm 73.61$ )	87.81 ( $\pm 63.54$ )	135.77 ( $\pm 58.35$ )
		Female	123.54 ( $\pm 77.44$ )	47.89 ( $\pm 37.23$ )	43.91 ( $\pm 22.90$ )	49.88 ( $\pm 21.67$ )	49.27 ( $\pm 14.27$ )	85.56 ( $\pm 76.27$ )	96.37 ( $\pm 62.54$ )	111.34 ( $\pm 52.31$ )
		Ind	-	10.22 ( $\pm 10.13$ )	11.94 ( $\pm 2.02$ )	9.27 ( $\pm 3.17$ )	-	14.01 ( $\pm 1.54$ )	11.37 ( $\pm 2.63$ )	-
2	TL	Male	140.13 ( $\pm 45.05$ )	126.36 ( $\pm 34.13$ )	118.33 ( $\pm 21.06$ )	118.97 ( $\pm 20.19$ )	139.17 ( $\pm 19.71$ )	135.84 ( $\pm 35.65$ )	142.61 ( $\pm 40.53$ )	170.11 ( $\pm 26.58$ )
		Female	154.28 ( $\pm 28.25$ )	135.78 ( $\pm 28.25$ )	119.71 ( $\pm 18.78$ )	122.08 ( $\pm 17.66$ )	127.18 ( $\pm 12.78$ )	140.32 ( $\pm 34.99$ )	146.70 ( $\pm 37.82$ )	157.43 ( $\pm 29.56$ )
		Ind.	-	75.38 ( $\pm 4.61$ )	82.03 ( $\pm 4.13$ )	72.12 ( $\pm 8.13$ )	-	78.63 ( $\pm 2.91$ )	74.67 ( $\pm 5.37$ )	-
3	SL	Male	103.46 ( $\pm 33.84$ )	97.30 ( $\pm 25.33$ )	91.91 ( $\pm 16.34$ )	94.54 ( $\pm 22.67$ )	105.77 ( $\pm 15.95$ )	105.51 ( $\pm 15.95$ )	111.33 ( $\pm 28.50$ )	131.28 ( $\pm 30.54$ )
		Female	113.63 ( $\pm 27.03$ )	104.35 ( $\pm 21.56$ )	93.55 ( $\pm 16.31$ )	94.62 ( $\pm 13.12$ )	98.22 ( $\pm 9.19$ )	108.60 ( $\pm 26.43$ )	113.97 ( $\pm 28.67$ )	121.07 ( $\pm 21.13$ )
		Ind.	-	58.00 ( $\pm 3.51$ )	63.09 ( $\pm 2.93$ )	56.19 ( $\pm 6.65$ )	-	61.01 ( $\pm 2.26$ )	58.89 ( $\pm 5.09$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthukadu	Palikat lake	Hyderabad
4	HL	Male	34.07 ( $\pm 10.29$ )	30.59 ( $\pm 8.49$ )	28.34 ( $\pm 4.97$ )	28.24 ( $\pm 4.31$ )	33.75 ( $\pm 4.40$ )	32.46 ( $\pm 8.27$ )	36.00 ( $\pm 11.67$ )	41.35 ( $\pm 6.66$ )
		Female	36.86 ( $\pm 8.09$ )	32.48 ( $\pm 6.73$ )	28.53 ( $\pm 4.06$ )	29.00 ( $\pm 3.74$ )	30.58 ( $\pm 2.68$ )	33.03 ( $\pm 8.00$ )	36.49 ( $\pm 10.35$ )	38.07 ( $\pm 7.15$ )
		Ind.	-	18.83 ( $\pm 1.12$ )	20.23 ( $\pm 1.04$ )	17.57 ( $\pm 1.73$ )	-	19.85 ( $\pm 1.15$ )	18.40 ( $\pm 1.71$ )	-
5	SNL	Male	14.58 ( $\pm 5.86$ )	13.06 ( $\pm 4.73$ )	11.71 ( $\pm 2.91$ )	11.58 ( $\pm 2.19$ )	14.67 ( $\pm 2.49$ )	13.76 ( $\pm 14.45$ )	15.92 ( $\pm 6.12$ )	19.70 ( $\pm 3.83$ )
		Female	15.99 ( $\pm 4.67$ )	14.08 ( $\pm 3.64$ )	11.90 ( $\pm 2.80$ )	11.91 ( $\pm 1.84$ )	12.90 ( $\pm 1.64$ )	14.05 ( $\pm 4.11$ )	16.15 ( $\pm 5.47$ )	17.53 ( $\pm 4.22$ )
		Ind.	-	7.02 ( $\pm 0.61$ )	7.59 ( $\pm 0.52$ )	6.56 ( $\pm 0.83$ )	-	7.53 ( $\pm 0.57$ )	7.74 ( $\pm 0.82$ )	-
6	LJL	Male	9.01 ( $\pm 2.83$ )	7.26 ( $\pm 1.85$ )	7.15 ( $\pm 1.64$ )	7.04 ( $\pm 1.10$ )	8.51 ( $\pm 1.04$ )	8.37 ( $\pm 2.25$ )	8.97 ( $\pm 2.04$ )	9.99 ( $\pm 9.98$ )
		Female	9.72 ( $\pm 2.04$ )	7.80 ( $\pm 1.76$ )	7.09 ( $\pm 1.21$ )	7.27 ( $\pm 1.01$ )	7.84 ( $\pm 0.87$ )	8.45 ( $\pm 2.17$ )	8.94 ( $\pm 1.70$ )	9.96 ( $\pm 1.46$ )
		Ind.	-	4.95 ( $\pm 0.34$ )	5.04 ( $\pm 0.51$ )	4.84 ( $\pm 0.41$ )	-	4.52 ( $\pm 0.36$ )	6.05 ( $\pm 0.57$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthakadu	Pulikat lake	Hyderabad
7	LD	Male	46.45 ( $\pm 14.33$ )	43.39 ( $\pm 12.08$ )	40.21 ( $\pm 7.34$ )	39.75 ( $\pm 6.48$ )	46.35 ( $\pm 6.18$ )	45.51 ( $\pm 11.51$ )	50.21 ( $\pm 15.46$ )	61.37 ( $\pm 12.38$ )
		Female	50.73 ( $\pm 11.38$ )	16.33 ( $\pm 9.46$ )	40.50 ( $\pm 5.85$ )	40.69 ( $\pm 5.24$ )	41.94 ( $\pm 5.25$ )	45.79 ( $\pm 11.43$ )	51.87 ( $\pm 14.20$ )	46.71 ( $\pm 21.00$ )
		Ind.	-	26.08 ( $\pm 1.84$ )	28.17 ( $\pm 1.27$ )	21.41 ( $\pm 2.77$ )	-	26.47 ( $\pm 1.93$ )	25.89 ( $\pm 1.93$ )	-
8.	LP	Male	35.01 ( $\pm 10.17$ )	31.44 ( $\pm 8.04$ )	29.13 ( $\pm 5.23$ )	29.84 ( $\pm 4.12$ )	35.51 ( $\pm 3.06$ )	33.43 ( $\pm 8.82$ )	38.99 ( $\pm 9.24$ )	42.83 ( $\pm 6.62$ )
		Female	37.91 ( $\pm 8.08$ )	33.44 ( $\pm 6.60$ )	28.98 ( $\pm 4.06$ )	30.42 ( $\pm 4.10$ )	32.72 ( $\pm 2.72$ )	33.83 ( $\pm 8.33$ )	39.07 ( $\pm 8.34$ )	39.38 ( $\pm 7.62$ )
		Ind.	-	19.65 ( $\pm 1.08$ )	20.65 ( $\pm 0.88$ )	18.90 ( $\pm 1.84$ )	-	20.13 ( $\pm 1.24$ )	20.04 ( $\pm 1.05$ )	-
9.	LVL	Male	43.30 ( $\pm 12.18$ )	37.66 ( $\pm 8.84$ )	35.78 ( $\pm 6.36$ )	35.36 ( $\pm 5.56$ )	42.56 ( $\pm 4.82$ )	40.51 ( $\pm 10.37$ )	43.06 ( $\pm 10.90$ )	49.25 ( $\pm 7.33$ )
		Female	47.08 ( $\pm 10.05$ )	40.37 ( $\pm 7.86$ )	35.54 ( $\pm 5.17$ )	36.12 ( $\pm 5.03$ )	38.13 ( $\pm 3.71$ )	41.56 ( $\pm 10.02$ )	43.84 ( $\pm 9.88$ )	45.40 ( $\pm 8.10$ )
		Ind.	-	24.38 ( $\pm 2.66$ )	24.60 ( $\pm 1.23$ )	22.60 ( $\pm 2.20$ )	-	24.02 ( $\pm 1.59$ )	24.89 ( $\pm 1.59$ )	-



Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthakadu	Pulikat lake	Hyderabad
10	LA	Male	57.16 ( $\pm 16.43$ )	51.75 ( $\pm 2.67$ )	47.94 ( $\pm 7.78$ )	48.60 ( $\pm 7.64$ )	57.67 ( $\pm 8.25$ )	55.53 ( $\pm 14.00$ )	58.06 ( $\pm 14.79$ )	69.42 ( $\pm 8.59$ )
		Female	62.61 ( $\pm 13.00$ )	55.54 ( $\pm 11.16$ )	48.82 ( $\pm 6.97$ )	50.31 ( $\pm 6.93$ )	52.17 ( $\pm 5.30$ )	56.53 ( $\pm 12.98$ )	59.16 ( $\pm 13.23$ )	64.34 ( $\pm 9.96$ )
		Ind.	-	32.30 ( $\pm 2.35$ )	33.63 ( $\pm 1.98$ )	30.52 ( $\pm 3.62$ )	-	32.57 ( $\pm 1.32$ )	34.00 ( $\pm 3.72$ )	-
11	CPL	Male	6.60 ( $\pm 2.34$ )	6.28 ( $\pm 2.16$ )	5.63 ( $\pm 1.25$ )	6.19 ( $\pm 1.18$ )	7.09 ( $\pm 1.05$ )	6.00 ( $\pm 1.96$ )	9.14 ( $\pm 2.68$ )	15.23 ( $\pm 10.15$ )
		Female	7.81 ( $\pm 2.22$ )	6.80 ( $\pm 2.09$ )	5.51 ( $\pm 1.28$ )	6.46 ( $\pm 1.17$ )	6.83 ( $\pm 0.83$ )	6.55 ( $\pm 3.27$ )	9.13 ( $\pm 2.20$ )	7.73 ( $\pm 1.47$ )
		Ind.	-	4.11 ( $\pm 1.48$ )	3.68 ( $\pm 0.54$ )	3.58 ( $\pm 0.64$ )	-	3.21 ( $\pm 0.18$ )	4.32 ( $\pm 0.49$ )	-
12	EYL	Male	10.71 ( $\pm 2.53$ )	10.41 ( $\pm 2.33$ )	9.53 ( $\pm 1.35$ )	9.65 ( $\pm 1.12$ )	10.94 ( $\pm 1.00$ )	10.73 ( $\pm 1.82$ )	12.67 ( $\pm 2.99$ )	12.11 ( $\pm 2.02$ )
		Female	11.42 ( $\pm 2.06$ )	11.04 ( $\pm 1.81$ )	9.75 ( $\pm 1.13$ )	6.47 ( $\pm 1.22$ )	10.30 ( $\pm 0.75$ )	11.02 ( $\pm 1.76$ )	12.45 ( $\pm 2.46$ )	11.63 ( $\pm 2.05$ )
		Ind.	-	6.92 ( $\pm 1.82$ )	7.55 ( $\pm 0.40$ )	6.47 ( $\pm 0.52$ )	-	7.11 ( $\pm 0.54$ )	7.30 ( $\pm 0.34$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Mathukadu	Puliket lake	Hyderabad
13	CFL	Male	32.67 ( $\pm 11.50$ )	29.21 ( $\pm 6.72$ )	27.67 ( $\pm 5.11$ )	27.26 ( $\pm 4.58$ )	32.47 ( $\pm 4.99$ )	32.16 ( $\pm 8.87$ )	35.01 ( $\pm 11.21$ )	40.67 ( $\pm 8.12$ )
		Female	35.71 ( $\pm 6.72$ )	31.66 ( $\pm 6.72$ )	28.15 ( $\pm 4.53$ )	27.99 ( $\pm 4.58$ )	28.93 ( $\pm 3.99$ )	33.64 ( $\pm 7.93$ )	36.11 ( $\pm 10.13$ )	37.17 ( $\pm 8.79$ )
		Ind.	-	17.41 ( $\pm 1.52$ )	19.42 ( $\pm 1.46$ )	16.02 ( $\pm 1.69$ )	-	18.53 ( $\pm 1.70$ )	17.86 ( $\pm 0.99$ )	-
14	EYD	Male	10.13 ( $\pm 2.11$ )	9.99 ( $\pm 2.60$ )	9.28 ( $\pm 1.32$ )	9.32 ( $\pm 1.13$ )	10.68 ( $\pm 1.02$ )	10.27 ( $\pm 1.66$ )	12.29 ( $\pm 2.42$ )	11.53 ( $\pm 1.78$ )
		Female	10.74 ( $\pm 1.77$ )	10.41 ( $\pm 1.66$ )	9.47 ( $\pm 0.96$ )	9.54 ( $\pm 1.19$ )	9.80 ( $\pm 1.51$ )	10.39 ( $\pm 0.66$ )	12.41 ( $\pm 1.61$ )	10.74 ( $\pm 1.84$ )
		Ind.	-	6.73 ( $\pm 0.40$ )	9.32 ( $\pm 0.48$ )	6.31 ( $\pm 0.54$ )	-	6.92 ( $\pm 0.65$ )	7.35 ( $\pm 0.27$ )	-
15	HD	Male	28.53 ( $\pm 7.73$ )	24.93 ( $\pm 6.97$ )	23.93 ( $\pm 4.15$ )	23.67 ( $\pm 3.74$ )	27.88 ( $\pm 3.84$ )	27.59 ( $\pm 6.38$ )	30.26 ( $\pm 8.79$ )	34.03 ( $\pm 5.97$ )
		Female	31.50 ( $\pm 3.59$ )	26.71 ( $\pm 5.45$ )	23.97 ( $\pm 3.58$ )	24.55 ( $\pm 3.26$ )	25.30 ( $\pm 2.82$ )	28.49 ( $\pm 6.60$ )	30.94 ( $\pm 8.07$ )	31.90 ( $\pm 6.10$ )
		Ind.	-	15.20 ( $\pm 0.91$ )	17.09 ( $\pm 1.08$ )	14.67 ( $\pm 1.69$ )	-	16.68 ( $\pm 1.42$ )	15.82 ( $\pm 1.66$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthukadu	Pulikat lake	Hyderabad
16	CHD	Male	11.75 ( $\pm 4.45$ )	10.85 ( $\pm 4.55$ )	9.61 ( $\pm 2.33$ )	9.94 ( $\pm 2.03$ )	12.56 ( $\pm 2.29$ )	11.34 ( $\pm 3.74$ )	14.15 ( $\pm 6.23$ )	16.83 ( $\pm 3.67$ )
		Female	12.58 ( $\pm 3.60$ )	11.68 ( $\pm 3.25$ )	0.49 ( $\pm 1.85$ )	9.90 ( $\pm 1.79$ )	10.90 ( $\pm 1.60$ )	11.48 ( $\pm 3.34$ )	14.59 ( $\pm 5.59$ )	15.68 ( $\pm 3.89$ )
		Ind.	-	5.31 ( $\pm 0.37$ )	6.03 ( $\pm 0.52$ )	5.88 ( $\pm 0.85$ )	-	5.44 ( $\pm 0.34$ )	6.90 ( $\pm 0.70$ )	-
17	GH	Male	61.64 ( $\pm 14.55$ )	56.98 ( $\pm 14.24$ )	53.64 ( $\pm 8.26$ )	54.79 ( $\pm 8.06$ )	59.64 ( $\pm 6.63$ )	58.67 ( $\pm 12.24$ )	65.81 ( $\pm 15.70$ )	79.62 ( $\pm 11.17$ )
		Female	66.23 ( $\pm 12.03$ )	60.57 ( $\pm 11.28$ )	53.82 ( $\pm 7.62$ )	56.07 ( $\pm 7.50$ )	55.89 ( $\pm 5.83$ )	60.70 ( $\pm 12.69$ )	67.26 ( $\pm 15.21$ )	75.47 ( $\pm 12.68$ )
		Ind.	-	34.70 ( $\pm 2.10$ )	37.67 ( $\pm 2.01$ )	34.60 ( $\pm 4.21$ )	-	36.45 ( $\pm 1.40$ )	37.66 ( $\pm 3.22$ )	
18	DIA 1	Male	60.17 ( $\pm 14.86$ )	56.28 ( $\pm 14.22$ )	52.95 ( $\pm 8.58$ )	53.53 ( $\pm 7.62$ )	59.32 ( $\pm 7.10$ )	57.67 ( $\pm 12.20$ )	64.26 ( $\pm 15.94$ )	78.35 ( $\pm 10.34$ )
		Female	64.99 ( $\pm 12.18$ )	59.81 ( $\pm 11.28$ )	53.12 ( $\pm 7.81$ )	55.34 ( $\pm 9.28$ )	55.59 ( $\pm 6.09$ )	59.70 ( $\pm 12.59$ )	66.01 ( $\pm 14.90$ )	73.93 ( $\pm 11.83$ )
		Ind.	-	33.91 ( $\pm 2.25$ )	33.05 ( $\pm 7.25$ )	33.75 ( $\pm 4.26$ )	-	35.39 ( $\pm 1.43$ )	36.90 ( $\pm 2.90$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthukadu	Palikot lake	Hyderabad
19	A <sub>1</sub> A <sub>2</sub>	Male	60.61 (± 13.85)	56.69 (± 14.15)	53.14 (± 8.13)	54.18 (± 7.82)	59.31 (± 6.72)	57.66 (± 12.20)	64.71 (± 15.42)	78.80 (± 11.15)
		Female	64.88 (± 11.50)	60.22 (± 11.29)	53.32 (± 7.00)	55.43 (± 7.05)	55.55 (± 5.79)	59.27 (± 12.44)	66.41 (± 14.76)	74.28 (± 12.22)
		Ind.	-	34.35 (± 2.25)	37.44 (± 1.99)	34.13 (± 4.22)	-	35.41 (± 1.40)	39.39 (± 3.64)	-
20	CPD	Male	19.56 (± 6.28)	16.64 (± 4.92)	15.91 (± 3.14)	16.11 (± 3.13)	17.98 (± 2.54)	18.07 (± 4.83)	20.18 (± 5.32)	22.97 (± 4.13)
		Female	20.93 (± 5.39)	17.97 (± 4.00)	15.96 (± 2.73)	16.50 (± 2.59)	15.96 (± 1.93)	18.83 (± 5.34)	20.41 (± 4.89)	21.33 (± 4.26)
		Ind.	-	9.58 (± 0.61)	10.80 (± 1.05)	9.04 (± 1.08)	-	10.57 (± 0.89)	10.91 (± 1.38)	-
21	POW	Male	13.14 (± 5.05)	12.15 (± 4.12)	10.99 (± 2.29)	11.24 (± 2.30)	13.83 (± 2.54)	12.73 (± 3.46)	13.69 (± 4.02)	18.67 (± 4.51)
		Female	14.33 (± 3.72)	12.97 (± 3.08)	11.42 (± 2.00)	11.46 (± 2.15)	12.15 (± 6.57)	13.34 (± 3.73)	14.24 (± 3.92)	16.84 (± 4.06)
		Ind.	-	6.57 (± 0.46)	7.69 (± 0.51)	-	-	7.43 (± 0.74)	7.46 (± 0.74)	-
22	LAW	Male	8.96 (± 3.34)	8.72 (± 3.01)	7.84 (± 1.79)	8.24 (± 1.84)	9.43 (± 1.77)	9.09 (± 2.34)	11.00 (± 3.21)	14.72 (± 3.50)
		Female	9.63 (± 2.46)	9.54 (± 2.35)	8.42 (± 1.85)	8.39 (± 1.67)	8.64 (± 1.68)	9.33 (± 2.35)	11.29 (± 2.66)	12.63 (± 2.72)
		Ind.	-	4.91 (± 0.49)	5.53 (± 0.54)	4.57 (± 0.67)	-	4.92 (± 0.43)	6.39 (± 0.75)	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthukadu	Pulikat Lake	Hyderabad
23	PP	Male	22.33 ( $\pm 6.37$ )	16.87 ( $\pm 5.33$ )	16.74 ( $\pm 3.57$ )	16.40 ( $\pm 3.33$ )	19.68 ( $\pm 4.24$ )	19.79 ( $\pm 5.74$ )	19.51 ( $\pm 5.50$ )	24.69 ( $\pm 5.89$ )
		Female	24.79 ( $\pm 6.23$ )	17.74 ( $\pm 3.92$ )	16.77 ( $\pm 3.38$ )	16.71 ( $\pm 3.56$ )	18.60 ( $\pm 4.58$ )	20.77 ( $\pm 6.64$ )	19.96 ( $\pm 4.93$ )	22.91 ( $\pm 4.50$ )
		Ind.	-	9.63 ( $\pm 0.57$ )	11.01 ( $\pm 0.62$ )	8.73 ( $\pm 1.13$ )	-	11.29 ( $\pm 0.60$ )	10.65 ( $\pm 0.76$ )	-
24	GB	Male	28.24 ( $\pm 10.35$ )	18.40 ( $\pm 5.66$ )	18.62 ( $\pm 4.45$ )	18.69 ( $\pm 4.42$ )	20.99 ( $\pm 4.46$ )	28.37 ( $\pm 10.59$ )	21.77 ( $\pm 1.16$ )	27.13 ( $\pm 6.85$ )
		Female	30.76 ( $\pm 8.02$ )	19.59 ( $\pm 4.50$ )	18.51 ( $\pm 3.67$ )	19.27 ( $\pm 3.99$ )	20.16 ( $\pm 4.14$ )	23.44 ( $\pm 8.85$ )	22.27 ( $\pm 5.16$ )	24.53 ( $\pm 5.00$ )
		Ind.	-	10.45 ( $\pm 0.54$ )	11.93 ( $\pm 0.72$ )	9.70 ( $\pm 1.12$ )	-	11.87 ( $\pm 0.44$ )	12.41 ( $\pm 2.30$ )	-
25	ICW	Male	13.13 ( $\pm 4.53$ )	12.01 ( $\pm 3.55$ )	11.25 ( $\pm 2.29$ )	11.06 ( $\pm 2.10$ )	13.59 ( $\pm 3.88$ )	12.60 ( $\pm 3.31$ )	14.27 ( $\pm 3.55$ )	17.69 ( $\pm 3.28$ )
		Female	14.25 ( $\pm 3.51$ )	12.86 ( $\pm 2.82$ )	11.12 ( $\pm 2.04$ )	11.36 ( $\pm 1.79$ )	12.23 ( $\pm 1.29$ )	13.07 ( $\pm 3.45$ )	14.67 ( $\pm 3.17$ )	16.31 ( $\pm 3.86$ )
		Ind.	-	6.74 ( $\pm 0.63$ )	7.70 ( $\pm 0.53$ )	6.93 ( $\pm 0.72$ )	-	7.33 ( $\pm 0.73$ )	8.02 ( $\pm 0.54$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Nuthukadu	Pulikat lake	Hyderabad
26.	CPB	Male	5.63 ( $\pm 2.58$ )	4.72 ( $\pm 1.57$ )	4.34 ( $\pm 4.45$ )	4.45 ( $\pm 1.19$ )	5.57 ( $\pm 0.91$ )	4.99 ( $\pm 1.89$ )	7.29 ( $\pm 1.02$ )	7.64 ( $\pm 1.89$ )
		Female	6.33 ( $\pm 2.07$ )	5.27 ( $\pm 1.41$ )	4.32 ( $\pm 1.12$ )	4.51 ( $\pm 0.93$ )	5.17 ( $\pm 0.89$ )	5.48 ( $\pm 2.13$ )	7.48 ( $\pm 1.07$ )	6.88 ( $\pm 1.70$ )
		Ind.	-	2.61 ( $\pm 0.30$ )	2.70 ( $\pm 0.45$ )	2.40 ( $\pm 0.41$ )	-	2.31 ( $\pm 0.27$ )	4.17 ( $\pm 0.72$ )	-
27.	LJW	Male	8.19 ( $\pm 3.39$ )	7.70 ( $\pm 2.76$ )	7.15 ( $\pm 1.45$ )	7.34 ( $\pm 1.76$ )	9.17 ( $\pm 1.65$ )	8.40 ( $\pm 2.37$ )	9.69 ( $\pm 2.52$ )	11.68 ( $\pm 2.49$ )
		Female	10.01 ( $\pm 2.98$ )	8.75 ( $\pm 2.35$ )	7.42 ( $\pm 1.41$ )	7.52 ( $\pm 1.53$ )	8.27 ( $\pm 1.22$ )	8.74 ( $\pm 2.92$ )	9.99 ( $\pm 2.23$ )	10.90 ( $\pm 2.82$ )
		Ind.	-	4.51 ( $\pm 0.38$ )	4.78 ( $\pm 0.36$ )	4.63 ( $\pm 0.41$ )	-	4.41 ( $\pm 0.24$ )	6.36 ( $\pm 0.73$ )	-
28.	PH	Male	30.71 ( $\pm 7.95$ )	27.38 ( $\pm 6.98$ )	26.67 ( $\pm 4.32$ )	26.53 ( $\pm 4.26$ )	30.12 ( $\pm 3.66$ )	29.70 ( $\pm 6.21$ )	32.41 ( $\pm 32.41$ )	37.63 ( $\pm 7.40$ )
		Female	32.97 ( $\pm 6.36$ )	29.74 ( $\pm 5.61$ )	26.29 ( $\pm 4.18$ )	26.84 ( $\pm 3.74$ )	27.63 ( $\pm 2.90$ )	30.50 ( $\pm 6.51$ )	33.25 ( $\pm 7.53$ )	35.51 ( $\pm 7.23$ )
		Ind.	-	17.24 ( $\pm 1.65$ )	19.32 ( $\pm 1.26$ )	18.84 ( $\pm 1.34$ )	-	19.03 ( $\pm 1.21$ )	17.79 ( $\pm 1.25$ )	-

Sl. No.	Character	Sex	Cochin	Mangalore	Karwar	Goa	Pondicherry	Mathakadu	Pulikat Lake	Hyderabad
29	VH	Male	23.60 ( $\pm 6.57$ )	21.39 ( $\pm 6.37$ )	21.33 ( $\pm 3.82$ )	21.62 ( $\pm 4.09$ )	22.22 ( $\pm 2.81$ )	23.37 ( $\pm 2.81$ )	25.99 ( $\pm 5.68$ )	26.72 ( $\pm 4.02$ )
		Female	25.48 ( $\pm 5.50$ )	23.21 ( $\pm 5.21$ )	20.99 ( $\pm 3.35$ )	21.84 ( $\pm 3.63$ )	20.20 ( $\pm 2.31$ )	24.25 ( $\pm 4.96$ )	26.49 ( $\pm 5.15$ )	25.00 ( $\pm 5.53$ )
		Ind.	-	12.76 ( $\pm 0.88$ )	14.69 ( $\pm 0.97$ )	12.64 ( $\pm 1.65$ )	-	14.24 ( $\pm 1.08$ )	14.35 ( $\pm 1.27$ )	-
30	LDPR	Male	22.32 ( $\pm 11.89$ )	19.44 ( $\pm 8.49$ )	19.18 ( $\pm 5.42$ )	18.56 ( $\pm 5.13$ )	23.57 ( $\pm 5.60$ )	24.83 ( $\pm 9.41$ )	24.94 ( $\pm 9.79$ )	34.74 ( $\pm 9.06$ )
		Female	25.77 ( $\pm 10.69$ )	21.39 ( $\pm 6.21$ )	19.12 ( $\pm 4.87$ )	18.56 ( $\pm 4.44$ )	19.41 ( $\pm 4.06$ )	25.48 ( $\pm 8.85$ )	26.09 ( $\pm 8.82$ )	30.64 ( $\pm 9.50$ )
		Ind.	-	9.75 ( $\pm 1.02$ )	11.09 ( $\pm 1.28$ )	8.61 ( $\pm 1.59$ )	-	10.10 ( $\pm 1.20$ )	9.34 ( $\pm 1.03$ )	-
31	LAPR	Male	20.32 ( $\pm 8.95$ )	19.10 ( $\pm 7.55$ )	18.12 ( $\pm 4.89$ )	17.68 ( $\pm 4.85$ )	23.00 ( $\pm 4.95$ )	22.94 ( $\pm 7.53$ )	23.82 ( $\pm 9.56$ )	31.70 ( $\pm 8.72$ )
		Female	23.68 ( $\pm 8.98$ )	20.77 ( $\pm 5.81$ )	18.05 ( $\pm 4.24$ )	17.80 ( $\pm 4.60$ )	19.06 ( $\pm 3.78$ )	23.39 ( $\pm 7.38$ )	24.93 ( $\pm 8.65$ )	28.41 ( $\pm 8.46$ )
		Ind.	-	9.53 ( $\pm 0.72$ )	10.88 ( $\pm 1.17$ )	8.32 ( $\pm 1.38$ )	-	10.54 ( $\pm 1.51$ )	8.91 ( $\pm 0.65$ )	

TABLE - 2 MEAN AND STANDARD DEVIATION OF DIFFERENT CHARACTERS IN SEX POOLED SAMPLES OF ETROPIUS SURATENSIS IN DIFFERENT LOCALITIES.

Sl. Char- No. cter	LOCALITY							
	Cochin	Manga- lore	Karwar	Goe	Pondi- cherry	Muthu- kadu	Pulikat lake	Hyderabad
1 WT	106.93 ( $\pm 138.08$ )	73.65 ( $\pm 45.91$ )	45.24 ( $\pm 24.96$ )	47.84 ( $\pm 22.60$ )	59.30 ( $\pm 26.76$ )	80.31 ( $\pm 74.00$ )	76.52 ( $\pm 66.31$ )	106.36 ( $\pm 62.89$ )
2 TL	140.87 ( $\pm 44.35$ )	139.73 ( $\pm 28.95$ )	120.04 ( $\pm 19.37$ )	119.87 ( $\pm 19.18$ )	132.74 ( $\pm 19.11$ )	136.77 ( $\pm 35.50$ )	131.46 ( $\pm 44.00$ )	151.60 ( $\pm 37.50$ )
3 SL	108.77 ( $\pm 33.25$ )	107.38 ( $\pm 21.41$ )	93.53 ( $\pm 15.90$ )	94.17 ( $\pm 18.84$ )	101.62 ( $\pm 14.63$ )	106.03 ( $\pm 27.79$ )	102.59 ( $\pm 33.36$ )	117.30 ( $\pm 27.47$ )
4 HL	34.08 ( $\pm 10.01$ )	33.53 ( $\pm 7.25$ )	28.65 ( $\pm 4.42$ )	28.46 ( $\pm 4.11$ )	32.09 ( $\pm 4.31$ )	32.51 ( $\pm 8.14$ )	32.94 ( $\pm 11.91$ )	37.13 ( $\pm 8.68$ )
5 SNL	14.58 ( $\pm 5.62$ )	14.64 ( $\pm 4.06$ )	11.93 ( $\pm 2.44$ )	11.67 ( $\pm 2.06$ )	13.75 ( $\pm 2.45$ )	13.77 ( $\pm 4.42$ )	14.49 ( $\pm 6.02$ )	17.24 ( $\pm 4.86$ )
6 LVL	9.03 ( $\pm 2.64$ )	7.96 ( $\pm 1.77$ )	7.19 ( $\pm 1.41$ )	7.11 ( $\pm 1.07$ )	8.14 ( $\pm 1.09$ )	8.24 ( $\pm 2.23$ )	8.44 ( $\pm 1.97$ )	9.25 ( $\pm 2.04$ )
7 LD	46.66 ( $\pm 13.94$ )	47.73 ( $\pm 10.24$ )	40.67 ( $\pm 6.45$ )	40.01 ( $\pm 5.98$ )	44.03 ( $\pm 6.51$ )	45.73 ( $\pm 11.53$ )	46.30 ( $\pm 16.34$ )	50.98 ( $\pm 18.08$ )
8 LP	34.96 ( $\pm 10.11$ )	34.31 ( $\pm 7.03$ )	29.29 ( $\pm 4.55$ )	29.98 ( $\pm 4.16$ )	34.03 ( $\pm 3.94$ )	33.37 ( $\pm 8.61$ )	35.40 ( $\pm 10.75$ )	38.56 ( $\pm 8.79$ )
9 LVL	43.42 ( $\pm 12.17$ )	41.47 ( $\pm 7.63$ )	35.96 ( $\pm 5.62$ )	35.56 ( $\pm 3.35$ )	40.30 ( $\pm 5.16$ )	40.69 ( $\pm 10.24$ )	39.97 ( $\pm 11.65$ )	44.83 ( $\pm 9.71$ )



Sl. No.	Character	LOCALITY							
		Cochin	Mangalore	Karwar	Goa	Pondicherry	Muthukadu	Palikat lake	Hyderabad
10	LA	57.53 ( $\pm 16.16$ )	56.98 ( $\pm 10.93$ )	48.76 ( $\pm 7.18$ )	49.18 ( $\pm 7.39$ )	54.61 ( $\pm 8.56$ )	55.42 ( $\pm 13.62$ )	54.10 ( $\pm 15.50$ )	62.55 ( $\pm 13.29$ )
11	CPL	6.85 ( $\pm 2.43$ )	7.01 ( $\pm 2.12$ )	5.63 ( $\pm 1.24$ )	6.27 ( $\pm 1.19$ )	6.94 ( $\pm 0.98$ )	6.17 ( $\pm 2.13$ )	8.21 ( $\pm 2.87$ )	7.52 ( $\pm 2.04$ )
12	EYL	10.71 ( $\pm 2.52$ )	11.33 ( $\pm 1.90$ )	9.71 ( $\pm 1.20$ )	9.72 ( $\pm 1.25$ )	10.59 ( $\pm 1.02$ )	10.80 ( $\pm 1.81$ )	11.53 ( $\pm 3.20$ )	11.11 ( $\pm 2.47$ )
13	CPL	37.70 ( $\pm 11.15$ )	32.58 ( $\pm 7.33$ )	28.14 ( $\pm 4.71$ )	27.46 ( $\pm 5.35$ )	30.63 ( $\pm 5.11$ )	32.49 ( $\pm 8.51$ )	32.13 ( $\pm 11.82$ )	35.72 ( $\pm 10.37$ )
14	EYD	10.09 ( $\pm 2.19$ )	10.73 ( $\pm 1.70$ )	9.44 ( $\pm 1.12$ )	9.38 ( $\pm 1.18$ )	10.23 ( $\pm 1.03$ )	10.28 ( $\pm 1.61$ )	11.38 ( $\pm 2.83$ )	10.50 ( $\pm 2.21$ )
15	HD	28.73 ( $\pm 8.15$ )	27.49 ( $\pm 5.89$ )	24.15 ( $\pm 3.75$ )	23.98 ( $\pm 3.56$ )	26.52 ( $\pm 3.85$ )	27.80 ( $\pm 6.50$ )	27.85 ( $\pm 9.36$ )	30.46 ( $\pm 7.80$ )
16	CHD	11.68 ( $\pm 4.26$ )	12.28 ( $\pm 3.79$ )	9.64 ( $\pm 2.07$ )	9.89 ( $\pm 1.91$ )	11.71 ( $\pm 2.25$ )	11.30 ( $\pm 3.58$ )	12.98 ( $\pm 5.96$ )	14.92 ( $\pm 6.44$ )
17	GH	61.42 ( $\pm 15.17$ )	62.50 ( $\pm 11.58$ )	54.16 ( $\pm 7.66$ )	55.16 ( $\pm 7.85$ )	57.57 ( $\pm 7.01$ )	59.15 ( $\pm 12.55$ )	61.07 ( $\pm 17.73$ )	71.97 ( $\pm 16.62$ )
18	D <sub>1</sub> A <sub>1</sub>	60.06 ( $\pm 15.35$ )	61.76 ( $\pm 11.56$ )	53.48 ( $\pm 7.92$ )	54.15 ( $\pm 8.45$ )	57.24 ( $\pm 7.37$ )	58.14 ( $\pm 12.51$ )	59.82 ( $\pm 17.51$ )	70.72 ( $\pm 16.01$ )
19	A <sub>1</sub> A <sub>2</sub>	60.25 ( $\pm 14.65$ )	62.16 ( $\pm 11.54$ )	53.65 ( $\pm 7.59$ )	54.54 ( $\pm 7.54$ )	57.23 ( $\pm 7.05$ )	57.95 ( $\pm 12.43$ )	60.35 ( $\pm 17.14$ )	71.08 ( $\pm 16.39$ )
20	CPD	19.39 ( $\pm 6.27$ )	18.51 ( $\pm 4.19$ )	16.08 ( $\pm 2.87$ )	16.19 ( $\pm 2.94$ )	16.93 ( $\pm 2.64$ )	18.27 ( $\pm 5.05$ )	18.57 ( $\pm 5.74$ )	20.44 ( $\pm 5.37$ )

Sl. No.	Character	LOCALITY							
		Cochin	Mangalore	Karwar	Goa	Pondicherry	Mathamkodu	Pulikat lake	Hyderabad
21	PCW	13.11 ( $\pm 4.47$ )	13.55 ( $\pm 8.39$ )	11.32 ( $\pm 2.32$ )	11.30 ( $\pm 2.22$ )	12.97 ( $\pm 2.30$ )	12.90 ( $\pm 3.58$ )	12.77 ( $\pm 4.30$ )	16.21 ( $\pm 5.19$ )
22	LAW	8.93 ( $\pm 3.10$ )	9.86 ( $\pm 2.52$ )	8.21 ( $\pm 1.81$ )	8.28 ( $\pm 1.75$ )	9.00 ( $\pm 1.81$ )	9.12 ( $\pm 2.37$ )	10.28 ( $\pm 3.16$ )	12.54 ( $\pm 3.93$ )
23	PF	22.40 ( $\pm 7.00$ )	18.63 ( $\pm 4.24$ )	16.89 ( $\pm 3.42$ )	16.45 ( $\pm 3.45$ )	19.01 ( $\pm 4.54$ )	20.03 ( $\pm 6.17$ )	18.14 ( $\pm 5.67$ )	22.12 ( $\pm 6.10$ )
24	GB	28.02 ( $\pm 10.19$ )	20.44 ( $\pm 4.62$ )	18.73 ( $\pm 4.01$ )	18.83 ( $\pm 4.27$ )	20.43 ( $\pm 4.49$ )	23.19 ( $\pm 9.83$ )	20.32 ( $\pm 5.90$ )	23.97 ( $\pm 6.95$ )
25	LOW	13.11 ( $\pm 4.35$ )	13.33 ( $\pm 2.95$ )	11.29 ( $\pm 2.12$ )	11.15 ( $\pm 1.96$ )	12.87 ( $\pm 1.89$ )	12.72 ( $\pm 3.36$ )	13.25 ( $\pm 3.90$ )	15.54 ( $\pm 4.52$ )
26	CPB	5.75 ( $\pm 2.38$ )	5.40 ( $\pm 1.40$ )	4.38 ( $\pm 1.17$ )	4.45 ( $\pm 1.07$ )	5.34 ( $\pm 0.97$ )	5.15 ( $\pm 2.00$ )	6.82 ( $\pm 1.50$ )	6.60 ( $\pm 2.10$ )
27	LJW	9.09 ( $\pm 3.27$ )	5.91 ( $\pm 2.43$ )	7.37 ( $\pm 1.38$ )	7.38 ( $\pm 1.66$ )	8.70 ( $\pm 1.59$ )	8.47 ( $\pm 2.62$ )	9.23 ( $\pm 2.46$ )	10.39 ( $\pm 3.07$ )
28	PH	30.71 ( $\pm 7.86$ )	30.34 ( $\pm 5.84$ )	26.66 ( $\pm 4.17$ )	26.56 ( $\pm 4.05$ )	28.79 ( $\pm 3.79$ )	29.86 ( $\pm 6.38$ )	29.98 ( $\pm 9.03$ )	33.88 ( $\pm 8.87$ )
29	WH	23.55 ( $\pm 6.65$ )	23.72 ( $\pm 5.65$ )	21.34 ( $\pm 3.48$ )	21.65 ( $\pm 3.86$ )	21.16 ( $\pm 2.96$ )	23.59 ( $\pm 5.03$ )	24.00 ( $\pm 6.65$ )	23.98 ( $\pm 5.95$ )
30	LDPR	22.81 ( $\pm 11.65$ )	22.32 ( $\pm 7.14$ )	19.39 ( $\pm 5.03$ )	18.45 ( $\pm 4.81$ )	21.50 ( $\pm 5.46$ )	24.86 ( $\pm 9.20$ )	22.44 ( $\pm 10.38$ )	29.23 ( $\pm 11.35$ )
31	LAPR	20.84 ( $\pm 9.21$ )	21.73 ( $\pm 6.33$ )	18.30 ( $\pm 4.47$ )	17.62 ( $\pm 4.73$ )	21.03 ( $\pm 4.97$ )	22.94 ( $\pm 7.47$ )	21.39 ( $\pm 10.12$ )	26.89 ( $\pm 10.44$ )

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[illegible]



CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS SURATENSIS IN KARWAR

No	Character	WT	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CPL	EYL	HD	CHD	GH	D1A1	A1A2	CPD	POW	LAW	PP	GB	IOW	CPB	LJW	PH	VH	LDPR	LAPR
1	WT	1																														
2	TL	0.973	1																													
3	SL	0.969	0.988	1																												
4	HL	0.975	0.978	0.972	1																											
5	SNL	0.963	0.980	0.967	0.984	1																										
6	LJL	0.894	0.892	0.896	0.984	0.895	1																									
7	LD	0.972	0.989	0.977	0.984	0.984	0.972	1																								
8	LP	0.971	0.977	0.969	0.977	0.991	0.974	0.987	1																							
9	LVL	0.956	0.974	0.972	0.972	0.974	0.972	0.972	0.972	1																						
10	LA	0.956	0.984	0.974	0.984	0.959	0.977	0.977	0.977	0.977	1																					
11	CPL	0.794	0.822	0.941	0.822	0.935	0.967	0.934	0.974	0.966	0.931	1																				
12	EYL	0.920	0.941	0.983	0.946	0.979	0.975	0.946	0.942	0.943	0.982	0.949	1																			
13	CPL	0.958	0.967	0.934	0.974	0.966	0.931	0.926	0.928	0.973	0.956	0.836	0.889	0.897	0.962	0.904	0.899	0.972	0.923	0.899	0.936	0.934	0.963	0.954	0.951	0.955	0.953	0.953	0.953	0.953	0.953	
14	EYL	0.933	0.967	0.934	0.974	0.966	0.931	0.926	0.928	0.973	0.956	0.836	0.889	0.897	0.962	0.904	0.899	0.972	0.923	0.899	0.936	0.934	0.963	0.954	0.951	0.955	0.953	0.953	0.953	0.953	0.953	
15	HD	0.968	0.979	0.975	0.946	0.942	0.943	0.982	0.949	0.832	0.881	0.899	0.972	0.923	0.899	0.936	0.934	0.963	0.954	0.951	0.955	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	
16	CHD	0.970	0.975	0.946	0.942	0.943	0.982	0.949	0.832	0.881	0.899	0.972	0.923	0.899	0.936	0.934	0.963	0.954	0.951	0.955	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953	
17	GH	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	0.915	0.914	
18	D1A1	0.914	0.915	0.914	0.915	0.914	0.915	0.914																								

TABLE - 6 CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS SURATENSIS IN GOA

NO	Character	WT	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CPL	EYD	HD	CHD	GH	D1A1	A1A2	CPD	POW	LAW	PP	GB	IQW	CPB	LJW	PH	VH	LDPR	LAPR
1	WT	1	0.922	0.878	0.962	0.946	0.875	0.947	0.852	0.960	0.961	0.810	0.920	0.928	0.892	0.910	0.914	0.957	0.798	0.922	0.961	0.969	0.938	0.927	0.917	0.952	0.926	0.950	0.954	0.965	0.920	0.907
2	TL		1	0.878	0.991	0.970	0.922	0.983	0.885	0.969	0.989	0.827	0.955	0.942	0.937	0.911	0.921	0.963	0.815	0.929	0.974	0.964	0.924	0.885	0.879	0.960	0.906	0.955	0.977	0.974	0.949	0.939
3	SL			1	0.872	0.871	0.819	0.877	0.748	0.847	0.849	0.571	0.944	0.812	0.807	0.837	0.820	0.826	0.701	0.803	0.934	0.867	0.831	0.727	0.739	0.853	0.825	0.850	0.845	0.865	0.861	0.840
4	HL				1	0.976	0.929	0.987	0.891	0.965	0.983	0.919	0.959	0.946	0.943	0.904	0.911	0.952	0.809	0.915	0.970	0.955	0.907	0.877	0.867	0.949	0.894	0.943	0.869	0.967	0.844	0.937
5	SNL					1	0.909	0.975	0.872	0.950	0.964	0.804	0.920	0.920	0.907	0.893	0.902	0.944	0.804	0.914	0.961	0.936	0.875	0.850	0.834	0.941	0.896	0.919	0.949	0.955	0.933	0.923
6	LJL						1	0.920	0.861	0.871	0.911	0.708	0.897	0.881	0.980	0.850	0.823	0.864	0.690	0.827	0.893	0.863	0.825	0.809	0.785	0.849	0.811	0.865	0.886	0.885	0.884	0.677
7	LD							1	0.890	0.954	0.972	0.785	0.951	0.920	0.936	0.893	0.887	0.945	0.810	0.904	0.960	0.944	0.894	0.841	0.840	0.949	0.882	0.923	0.954	0.958	0.940	0.932
8	LP								1	0.802	0.870	0.767	0.884	0.841	0.882	0.775	0.790	0.856	0.736	0.818	0.874	0.855	0.799	0.776	0.749	0.840	0.790	0.852	0.869	0.951	0.831	0.843
9	LVL									1	0.963	0.800	0.903	0.904	0.885	0.893	0.900	0.933	0.793	0.898	0.949	0.843	0.903	0.890	0.878	0.939	0.891	0.932	0.939	0.945	0.915	0.899
10	LA										1	0.835	0.929	0.922	0.913	0.897	0.904	0.945	0.794	0.990	0.960	0.944	0.899	0.885	0.879	0.932	0.907	0.936	0.965	0.961	0.929	0.917
11	CPL											1	0.792	0.780	0.772	0.762	0.911	0.781	0.667	0.785	0.821	0.821	0.761	0.783	0.768	0.789	0.805	0.832	0.838	0.797	0.773	0.760
12	EYL												1	0.928	0.964	0.865	0.876	0.924	0.817	0.880	0.942	0.936	0.889	0.820	0.946	0.917	0.850	0.920	0.945	0.925	0.920	0.928
13	CPL													1	0.900	0.881	0.874	0.931	0.781	0.903	0.940	0.922	0.891	0.843	0.839	0.899	0.861	0.930	0.934	0.941	0.914	0.916
14	EYD														1	0.841	0.852	0.902	0.826	0.870	0.909	0.909	0.858	0.814	0.801	0.903	0.827	0.895	0.924	0.906	0.894	0.908
15	HD															1	0.845	0.895	0.745	0.934	0.890	0.910	0.867	0.829	0.828	0.888	0.837	0.908	0.892	0.890	0.869	0.859
16	CHD																1	0.882	0.718	0.849	0.907	0.934	0.869	0.826	0.833	0.902	0.822	0.920	0.935	0.928	0.915	0.897
17	GH																	1	0.867	0.971	0.958	0.946	0.925	0.888	0.872	0.946	0.885	0.933	0.961	0.958	0.905	0.909
18	D1A1																		1	0.860	0.829	0.792	0.758	0.723	0.710	0.793	0.752	0.788	0.815	0.794	0.757	0.767
19	A1A2																			1	0.922	0.919	0.897	0.843	0.831	0.921	0.864	0.916	0.932	0.921	0.888	0.873
20	CPD																				1	0.956	0.905	0.895	0.899	0.942	0.899	0.949	0.959	0.956	0.925	0.918
21	POW																					1	0.951	0.886	0.892	0.969	0.892	0.972	0.956	0.966	0.932	0.921
22	LAW																						1	0.856	0.861	0.943	0.867	0.958	0.923	0.929	0.895	0.892
23	PP																							1	0.957	0.862	0.967	0.863	0.870	0.871	0.812	0.800
24	GB																								1	0.863	0.847	0.879	0.862	0.860	0.799	0.784
26	IQW																									1	0.888	0.944	0.951	0.963	0.919	0.915
27	CPB																										1	0.902	0.905	0.912	0.836	0.932
28	LJW																											1	0.956	0.951	0.935	0.932
29	PH																												1	0.975	0.953	0.939
30	VH																													1	0.951	0.940
31	LDPR																														1	0.969
32	LAPR																															1

TABLE - 7 CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS SURATENSIS IN PONDICHERRY

No	Character	WT	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CPL	EYD	HD	CHD	GH	DIA1	A1A2	CPD	POW	LAW	PP	GB	IOW	CPB	LJW	PH	VH	LDPR	LAFR
1	WT	1	0.782	0.637	0.880	0.850	0.791	0.831	0.855	0.857	0.790	0.646	0.799	0.862	0.815	0.867	0.892	0.793	0.853	0.861	0.860	0.875	0.835	0.640	0.655	0.683	0.847	0.663	0.791	0.854	0.815	0.827
2	TL		1	0.967	0.961	0.943	0.911	0.942	0.925	0.921	0.918	0.732	0.889	0.930	0.874	0.950	0.911	0.944	0.945	0.946	0.931	0.907	0.762	0.666	0.700	0.951	0.782	0.883	0.953	0.929	0.902	0.870
3	SL			1	0.885	0.872	0.849	0.883	0.839	0.832	0.864	0.676	0.808	0.950	0.800	0.883	0.832	0.885	0.887	0.885	0.865	0.841	0.711	0.602	0.635	0.890	0.732	0.823	0.880	0.855	0.825	0.798
4	HL				1	0.980	0.923	0.958	0.970	0.954	0.927	0.745	0.925	0.945	0.935	0.966	0.938	0.949	0.947	0.952	0.953	0.932	0.737	0.678	0.720	0.969	0.765	0.869	0.970	0.942	0.921	0.880
5	SNL					1	0.891	0.942	0.961	0.938	0.897	0.765	0.911	0.926	0.921	0.951	0.930	0.920	0.921	0.923	0.929	0.924	0.742	0.643	0.685	0.940	0.767	0.877	0.959	0.933	0.914	0.854
6	LJL						1	0.916	0.902	0.908	0.863	0.727	0.855	0.904	0.864	0.914	0.886	0.923	0.921	0.925	0.876	0.881	0.740	0.608	0.657	0.893	0.661	0.810	0.905	0.886	0.844	0.837
7	LD							1	0.923	0.950	0.883	0.742	0.908	0.924	0.901	0.951	0.903	0.945	0.939	0.945	0.920	0.898	0.746	0.640	0.669	0.950	0.724	0.842	0.948	0.934	0.905	0.881
8	LP								1	0.940	0.906	0.715	0.913	0.904	0.902	0.926	0.891	0.918	0.917	0.920	0.914	0.880	0.795	0.627	0.679	0.921	0.774	0.821	0.945	0.917	0.899	0.863
9	LVL									1	0.873	0.707	0.895	0.912	0.892	0.930	0.883	0.894	0.892	0.898	0.891	0.888	0.728	0.632	0.679	0.914	0.748	0.834	0.944	0.918	0.904	0.882
10	LA										1	0.660	0.862	0.862	0.881	0.907	0.843	0.899	0.896	0.900	0.908	0.854	0.670	0.625	0.652	0.904	0.709	0.795	0.890	0.883	0.845	0.843
11	CPL											1	0.734	0.751	0.692	0.750	0.761	0.751	0.731	0.750	0.714	0.683	0.636	0.427	0.489	0.714	0.478	0.736	0.735	0.750	0.688	0.663
12	EYL												1	0.901	0.915	0.898	0.864	0.901	0.891	0.901	0.863	0.862	0.732	0.586	0.628	0.914	0.703	0.830	0.917	0.914	0.893	0.846
13	CPL													1	0.873	0.934	0.918	0.936	0.932	0.938	0.919	0.923	0.768	0.646	0.678	0.943	0.659	0.874	0.959	0.920	0.908	0.859
14	EYD														1	0.903	0.892	0.875	0.871	0.878	0.878	0.889	0.718	0.543	0.576	0.918	0.682	0.785	0.899	0.919	0.974	0.853
15	HD															1	0.947	0.948	0.955	0.954	0.937	0.729	0.764	0.670	0.711	0.757	0.749	0.886	0.954	0.928	0.908	0.873
16	CHD																1	0.924	0.931	0.929	0.946	0.920	0.770	0.661	0.687	0.932	0.744	0.903	0.928	0.908	0.891	0.845
17	GH																	1	0.993	0.998	0.937	0.889	0.779	0.664	0.682	0.953	0.733	0.863	0.947	0.932	0.898	0.862
18	DIA1																		1	0.995	0.936	0.901	0.784	0.679	0.697	0.947	0.749	0.871	0.944	0.925	0.895	0.849
19	A1A2																			1	0.937	0.896	0.785	0.669	0.688	0.956	0.736	0.870	0.949	0.932	0.902	0.863
20	CPD																				1	0.900	0.740	0.669	0.687	0.942	0.744	0.883	0.937	0.911	0.893	0.854
21	POW																					1	0.803	0.628	0.664	0.926	0.742	0.872	0.939	0.901	0.897	0.830
22	LAW																						1	0.456	0.491	0.777	0.649	0.830	0.811	0.793	0.773	0.734
23	PP																							1	0.928	0.667	0.504	0.580	0.648	0.600	0.620	0.535
24	GB																								1	0.682	0.569	0.608	0.677	0.603	0.620	0.547
25	IOW																									1	0.752	0.874	0.962	0.956	0.928	0.874
26	CPB																										1	0.756	0.781	0.735	0.721	0.620
27	LJW																											1	0.888	0.858	0.862	0.785
28	PH																												1	0.960	0.943	0.900
29	VH																													1	0.947	0.404
30	LDPR																														1	0.909
31	LAFR																															1

TABLE - 8

CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEI POOLED SAMPLES OF *ETROPLUS SURATENSIS* IN MUTHUKADI

MORPHOMETRIC CHARACTERS OF SEI POOLED SAMPLES OF <i>ETROPLUS SURATENSIS</i> IN MUTHUKADU																																
NO	Character	WT	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CPL	EYD	HD	CHD	GH	DIA1	A1A2	CPD	POW	LAW	PP	GB	ICW	CPB	LJW	PH	VH	LDPR	LAPR
1	WT	1	0.947	0.940	0.943	0.958	0.896	0.931	0.947	0.958	0.906	0.891	0.859	0.841	0.851	0.903	0.913	0.920	0.919	0.916	0.945	0.906	0.856	0.950	0.957	0.896	0.946	0.867	0.925	0.916	0.898	0.892
2	TL		1	0.995	0.990	0.986	0.956	0.992	0.992	0.990	0.972	0.953	0.943	0.988	0.943	0.974	0.980	0.985	0.985	0.985	0.988	0.978	0.945	0.978	0.936	0.980	0.969	0.946	0.980	0.978	0.969	0.972
3	SL			1	0.986	0.983	0.959	0.989	0.989	0.987	0.965	0.939	0.942	0.988	0.935	0.966	0.976	0.978	0.977	0.979	0.980	0.971	0.944	0.971	0.936	0.970	0.960	0.935	0.975	0.973	0.969	0.971
4	HL				1	0.988	0.955	0.992	0.995	0.990	0.975	0.946	0.945	0.976	0.944	0.967	0.977	0.974	0.973	0.973	0.986	0.968	0.937	0.980	0.941	0.969	0.968	0.948	0.983	0.964	0.959	0.974
5	SNL					1	0.935	0.981	0.985	0.989	0.966	0.932	0.920	0.979	0.923	0.943	0.969	0.967	0.967	0.967	0.980	0.955	0.930	0.968	0.944	0.954	0.962	0.922	0.976	0.947	0.946	0.956
6	LJL						1	0.959	0.960	0.941	0.928	0.896	0.954	0.949	0.922	0.946	0.939	0.945	0.940	0.947	0.946	0.941	0.909	0.944	0.881	0.933	0.912	0.913	0.942	0.934	0.938	0.954
7	LD							1	0.992	0.988	0.973	0.955	0.953	0.991	0.947	0.970	0.977	0.989	0.987	0.988	0.984	0.976	0.951	0.977	0.922	0.978	0.968	0.950	0.987	0.970	0.967	0.979
8	LP								1	0.990	0.970	0.942	0.944	0.980	0.943	0.970	0.978	0.978	0.976	0.977	0.980	0.967	0.939	0.976	0.940	0.970	0.955	0.941	0.979	0.968	0.969	0.975
9	LVL									1	0.964	0.935	0.925	0.982	0.920	0.957	0.946	0.976	0.976	0.975	0.980	0.961	0.929	0.983	0.957	0.958	0.974	0.930	0.979	0.970	0.954	0.950
10	LA										1	0.931	0.923	0.963	0.948	0.953	0.969	0.967	0.968	0.969	0.964	0.951	0.931	0.946	0.891	0.961	0.935	0.933	0.961	0.947	0.954	0.975
11	CPL											1	0.920	0.927	0.934	0.945	0.941	0.947	0.945	0.943	0.961	0.957	0.907	0.945	0.858	0.963	0.929	0.944	0.939	0.941	0.924	0.941
12	EYL												1	0.928	0.964	0.935	0.941	0.941	0.936	0.940	0.971	0.941	0.918	0.930	0.846	0.943	0.912	0.922	0.954	0.930	0.931	0.951
13	CHD													1	0.934	0.953	0.938	0.935	0.938	0.941	0.946	0.929	0.913	0.837	0.954	0.896	0.914	0.943	0.930	0.930	0.952	
14	EYD														1	0.972	0.966	0.966	0.965	0.973	0.973	0.932	0.958	0.896	0.974	0.944	0.947	0.957	0.970	0.956	0.965	
15	HD															1	0.971	0.971	0.971	0.942	0.976	0.969	0.946	0.896	0.974	0.942	0.932	0.975	0.959	0.965	0.972	
16	GH																1	0.998	0.997	0.978	0.973	0.951	0.962	0.899	0.977	0.962	0.949	0.982	0.965	0.963	0.970	
17	DIA1																	1	0.998	0.975	0.972	0.950	0.960	0.898	0.975	0.960	0.946	0.980	0.964	0.959	0.968	
18	A1A2																		1	0.972	0.973	0.954	0.958	0.892	0.975	0.956	0.943	0.979	0.954	0.964	0.972	
19	CPD																			1	0.957	0.952	0.870	0.986	0.935	0.946	0.963	0.963	0.950	0.962		
20	POW																				1	0.897	0.829	0.953	0.907	0.911	0.950	0.924	0.941	0.946		
21	LAW																					1	0.948	0.947	0.976	0.947	0.970	0.965	0.939	0.951		
22	PP																						1	0.873	0.948	0.854	0.920	0.926	0.888	0.885		
23	GB																							1	0.941	0.956	0.966	0.961	0.956	0.964		
24	ICW																								1	0.928	0.976	0.958	0.940	0.940		
25	CPB																									1	0.941	0.922	0.932	0.946		
26	LJW																										1	0.960	0.957	0.969		
27	PH																											1	0.968	0.962		
28	VH																												1	0.984		
29	LDPR																													1	0.984	
30	LAPR																														1	0.984



CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS SURATENSIS IN PULIKAT LAKE

[illegible]

CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS SURATE IN HYDERABAD

[illegible]

In Cochin, the mean body weight was observed to be 106.93 gms (minimum 4.9 gm and maximum 768.0 gms); in Mangalore 73.65gms (range 8.8 gm - 231.0 gms); in Karwar 45.24 gms (range 9.6-118.5 gms); in Goa, 47.84 gms (range 3.4-96.5 gms); in Pondicherry, 59.30 gms (range 5.5-117.2 gms); in Muthukadu, 80.31 gms (range 9.2-275.0 gms); in Pulikat lake, 76.52 gms (range 7.8-256.0 gms) and in Hyderabad 106.36 gms (range 12.2-238.0 gms) (Table - 2).

Correlation between body weight and all other 31 morphometric characters in pooled out sexes were positive and significant in all the localities (Tables 3-10).

#### Total Length (TL)

Significant differences were observed in respect of total length between sexes, as well as in the pooled out sexes in different localities (Tables 1 & 2). The total length of sex pooled samples varied between 58.0-310.00 mm, with mean total length of 140.87 mm in Cochin; 67.0-217.0mm with mean TL of 139.73 mm in Mangalore, 75.0-172.0 mm with mean TL of 120.04 mm in Karwar, 53.0-155.00 mm with mean TL of 119.87 mm in Goa, 63.0-170.00 mm with mean TL of 132.74 mm in Pondicherry, 74.2-210.00 mm with mean TL of 136.77 mm in Muthukadu, 67.5-225.0 mm with mean TL of 131.46 mm in Pulikat lake and 75.0-212.0 mm with mean TL of 151.60 mm in Hyderabad (Table 2).

Correlations were positive and significant between total length and all other characters for pooled out sexes studied in different localities (Table 3-10).

#### Standard length (SL)

Significant differences were observed in standard length (SL) between sexes as well as of pooled out sexes of the fish from different localities. (Tables 1 and 2). The mean standard length of sex pooled samples was observed to be 108.77 mm (46.0-234.0mm) in Cochin; 107.38 mm (range 52.5-162.00 mm) in Mangalore; 93.53 mm (range 58.5-135.0 mm) in Karwar; 94.17 mm (range 42.0-118.0 mm) in Goa, 101.62 mm (range 49.0-130.5 mm) in Pondicherry; 106.03 mm (range 58.1-168.0 mm) in Muthukadu; 102.59 mm (range 52.0-172.0 mm) in Pulikat lake and 117.3 mm (range 57.0-160.5 mm) in Hyderabad (Table-2).

Significant and positive correlations were observed between standard length and all the characters for pooled out sexes studied in different localities (Table 3-10).

#### Head Length (HL)

There were significant differences between sexes as well as in pooled out sexes in respect of head length in different localities (Tables 1 and 2). Head length varied in sex pooled samples from 15.2-73.0 mm (mean of 34.08 mm) in Cochin, 16.6-54.9 mm (mean 33.53 mm) in Mangalore, 19.0-14.1 mm (mean 28.65 mm) in Karwar, 13.4-36.0 mm (mean 28.46 mm) in Goa, 15.8-41.0 mm (mean 32.09 mm) in Pondicherry,

18.8-49.0 mm (mean 32.51 mm) in Muthukadu, 16.5-61.2 mm (mean 32.94)mm) in Pulikat lake and 19.8-51.8 mm (mean 37.13 mm) in Hyderabad (Table 2).

Correlations observed between head length and all the other characters studied were positive and significant for pooled out sexes in all the localities (Tables 3-10).

#### Snout length (SNL)

Significant differences were observed in respect of snout length in sexes and in pooled out sexes in different localities (Tables 1 & 2). The mean snout length of pooled samples was 14.59 mm (5.0-36.5mm) in Cochin, 14.64 mm (range 6.2-27.0) in Mangalore, 11.93 mm (range 7.0-18.9 mm) in Karwar, 11.67 mm (range 4.5-15.9 mm) in Goa, 13.75 mm (range 6.5-19.2 mm) in Pondicherry, 13.77 mm (range 6.9-24.0 mm) in Muthukadu, 14.49 mm (range 6.5-28.5 mm) in Pulikat lake and 17.24 mm (range 8.1-25.5 mm) in Hyderabad (Table 2).

There were positive and significant correlations between snout length and all the characters studied in pooled out sexes of different localities (Tables 3-10).

#### Lower Jaw length (LJL)

There was significant difference in respect of lower jaw length between sexes and pooled sex samples of different localities (Tables 1 and 2). Lower jaw length varied from

4.8-21.0 mm (mean 9.03) in Cochin, 4.7-13.5 mm (mean 7.96 mm) in Mangalore, 4.5-11.4 mm (mean 7.19 mm) in Karwar, 3.60-9.00 mm (mean 7.11 mm) in Goa, 4.50-10.00mm (mean 8.14) in Pondicherry, 4.00-12.10 mm (mean 8.24 mm) in Muthukadu, 5.20-15.20 mm (mean 8.44 mm) in Pulikat lake and 5.20-12.00 mm (mean 9.25 mm) in Hyderabad (Table 2).

Correlations were positive and significant between lower jaw length with all the characters studied in pooled out sexes in different localities (Tables 3-10).

#### Pre-dorsal distance (LD)

Significant differences were observed in respect of pre-dorsal distance between sexes and pooled out sexes in different localities (Table 1 & 2). The mean pre-dorsal distance in sex pooled samples observed to be 46.66 mm (range 20.5-99.0 mm) in Cochin, 47.73 mm (range 23.4-76.8 mm) in Mangalore, 40.68 mm (range 25.9-59.1 mm) in Karwar, 40.01 mm (range 18.9-52.0 mm) in Goa, 44.03 mm (range 20.8-56.9 mm) in Pondicherry, 45.73 mm (range 22.0-67.5 mm) in Muthukadu, 46.30 mm (range 22.5-91 mm) in Pulikat lake and 50.98 mm (range 25.0-74.0 mm) in Hyderabad (Table 2).

The correlation observed between predorsal distance and all the characters for pooled out sexes in different localities was positive and significant (Tables 3-10).

### Pre-pectoral distance (LP)

Differences were significant in respect of pre-pectoral distance in different sexes and pooled out sexes of different localities (Tables 1 and 2). The mean pre-pectoral distance in sex pooled samples varied between 16.8-72.0 mm (mean 34.96 mm) in Cochin, 17.1-52.2 mm (mean 34.31 mm) in Mangalore, 18.9-42.2 mm (mean 29.29 mm) in Karwar, 14.8-25.2 mm (mean 29.98 mm) in Goa, 17.4-42.2 mm (mean 34.03 mm) in Pondicherry, 18.0-51.5 mm (mean 33.39 mm) in Muthukadu, 18.8-58.0 mm (mean 35.40 mm) in Pulikat lake and 20.8-53.8 mm (mean 38.56 mm) in Hyderabad (Table 2).

Correlation observed in sex pooled samples between pre-pectoral distance and all the characters in different localities were positive and significant (Tables 3-10).

### Pre-ventral distance (LVL)

The mean pre-ventral distance in different sexes of different localities and in sex pooled samples of different localities showed significant differences (Tables 1 and 2). The mean pre-ventral distances observed in sex pooled samples were 43.42 mm (range 17.9-87.5 mm) in Cochin, 41.47 mm (range 20.8-59.4 mm) in Mangalore, 35.96 mm (range 22.3-52.3 mm) in Karwar, 35.56 mm (range 17.2-64.6 mm) in Goa, 40.30 mm (range 19.8-50.0 mm) in Pondicherry, 40.69 mm (range 22.0-64.0 mm) in Muthukadu, 39.97 mm (range 23.0-62.8 mm) in Pulikat lake

and 44.83 mm (range 26.2-60.8 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between pre-ventral distance and all the characters studied in different localities (Table 3-10).

#### Pre-anal distance (LA)

Mean pre-anal distance in different sexes as well as in sex pooled samples showed significant differences in different localities (Table 1 and 2). The range of pre-anal distance was recorded as 25.5-119.0 mm (mean 57.53 mm) in Cochin, 28.0-81.1 mm (mean 56.98 mm) in Mangalore, 31.2-66.1 mm (mean 48.73 mm) in Kannur, 22.6-60.2 mm (mean 49.18 mm) in Goa, 27.8-70.5 mm (mean 54.51 mm) in Pondicherry, 31.0-82.5 mm (mean 55.42 mm) in Kuthukadu, 29.5-87.0 mm (mean 54.10 mm) in Pulikat lake and 34.4-86.5 mm (mean 62.55 mm) in Hyderabad. (Table 2).

Correlations observed between pre-anal distance (LA) and all the characters studied in different localities were positive and significant.

#### Caudal peduncle length (CPL)

Differences were significant in respect of caudal peduncle length of samples of different sex and pooled out sexes in different localities (Tables 1 and 2). Mean caudal peduncle length was recorded as 6.85 mm (range 2.8-15.5 mm) in Cochin, 7.01 mm (range 3.8-14.1 mm) in Mangalore, 5.63 mm



(range 3.3-8.9 mm) in Karwar, 6.27 mm (range 2.8-7.0 mm) in Goa, 6.94 mm (range 3.5-8.3 mm) in Pondicherry, 6.17 mm (range 3.0-8.4 mm) in Muthukadu, 8.22 mm (range 4.2-17.1 mm) in Pulikat lake and 7.52 mm (range 4.0-12.8 mm) in Hyderabad.

Correlations were positive and significant between caudal peduncle length and all the characters in sex pooled samples of different localities (Tables 3-10).

#### Eye Diameter (Horizontal) (EYL)

The mean eye diameter (horizontal) (EYL) showed significant difference in samples of sexes as well as sex pooled samples in different localities (Tables 1 and 2). The range for EYL for sex pooled samples recorded was 5.9-20.0 mm (mean 10.71 mm) in Cochin, 6.2-16.1 mm (mean 11.33 mm) in Mangalore, 6.9-12.9 mm (mean 9.71 mm) in Karwar, 5.25-10.77 mm (mean 9.72 mm) in Goa, 5.2-12.0 mm (mean 10.59 mm) in Pondicherry, 6.0-13.2 mm (mean 10.80 mm) in Muthukadu, 7.5-18.7 mm (mean 11.53 mm) in Pulikat lake and 6.2-14.5 mm (mean 11.11 mm) in Hyderabad (Table 2).

Correlations observed between EYL and all the characters studied for pooled out sexes were positive and significant in different localities (Tables 3 — 10).

#### Caudal fin length (CFL)

Significant differences were observed in respect of caudal fin length in different sexes as well as sex pooled

samples of different localities (Tables 1 and 2). The range of caudal fin length in sex pooled samples recorded was 13.5-76.0 mm (mean 37.70 mm) in Cochin, 15.4-53.5 mm (mean 32.58mm) in Mangalore, 16.5-40.9 mm (mean 28.14 mm) in Karwar, 12.3-35.8 mm (mean 27.46 mm) in Goa, 13.0-39.0 mm (mean 30.63 mm) in Pondicherry, 16.2-53.0 mm (mean 32.49 mm) in Muthukadu, 17.2-58.9 mm (mean 32.13 mm) in Pulikat lake and 17.0-53.8 mm (mean 35.72 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between caudal fin length and all the characters studied in sex pooled samples of different localities (Tables 3-10).

#### Eye diameter (vertical) (EYD)

Mean eye diameter (vertical) showed differences in the samples of different sexes as well as pooled out sexes in different localities (Tables 1 and 2). The mean eye diameter recorded was 10.09 mm (range 5.0-17.0 mm) in Cochin, 10.73 mm (6.0-15.8 mm) in Mangalore, 9.44 mm (6.0-12.8 mm) in Karwar, 9.38 mm (4.9-11.2 mm) in Goa, 10.23 mm (5.8-12.0 mm) in Pondicherry, 10.28 mm (6.0-12.5 mm) in Muthukadu, 11.38 mm (7.2-17.9 mm) in Pulikat lake, and 10.50 mm (17.0-53.8 mm) in Hyderabad (Table 2).

Correlations observed between eye diameter (vertical) and all the characters of pooled sexes studied in different localities were positive and significant (Table 3 - 10).

Head depth (HD)

Differences were significant in respect of head depth in the samples of different sexes as well as pooled out sexes in different localities (Tables 1 and 2). Head depth varied in sex pooled samples between 12.4-56.0 mm (mean 28.73 mm) in Cochin, 13.2-44.2 mm (mean 27.49 mm) in Mangalore, 15.3-33.7 mm (mean 24.15 mm) in Karwar, 10.5-31.9 mm (mean 23.98 mm) in Goa, 12.5-35.5 mm (mean 26.52 mm) in Pondicherry, 15.0-38.5 mm (mean 27.80 mm) in Muthukadu, 14.5-47.2 mm (mean 27.85 mm) in Pulikat lake and 13.8 mm-43.00 mm (mean 30.46 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between head depth and all the characters studied in different localities (Tables 3-10).

Cheek depth (CMD)

Significant differences were observed in respect of cheek depth in the samples of different sexes as well as pooled out sexes in different localities (Table 1 and 2). The cheek depth varied between 4.8-28.1 mm (mean 11.66 mm) in Cochin, 4.9-24.0 mm (mean 12.28 mm) in Mangalore, 5.1-15.7 mm (mean 9.64 mm) in Karwar, 4.0-13.5 mm (mean 9.89 mm) in Goa, 4.0-16.5 mm (mean 11.71 mm) in Pondicherry, 4.9-18.0 mm (mean 11.39 mm) in Muthukadu, 6.2-30.0 mm (mean 12.98 mm) in Pulikat lake and 6.8-22.0 mm (mean 14.92 mm) in Hyderabad (Table-2).

Correlations between cheek depth and all the characters of pooled out sexes studied were positive and significant in different localities (Tables 3-10).

#### Greatest depth (GH)

Differences were significant in respect of greatest depth of different sexes as well as pooled out sexes in different sexes as well as pooled out sexes in different localities (Tables 1 and 2). The range for greatest depth in sex pooled samples recorded was 27.9-115.0 mm (mean 61.42 mm) in Cochin, 30.5-94.00 mm (mean 62.50 mm) in Mangalore, 35.8-68.5 mm (mean 54.16 mm) in Karwar, 24.5-67.5 mm (mean 55.16 mm) in Goa, 28.5-69.5 mm (mean 57.57 mm) in Pondicherry, 34.5-79.8 mm (mean 59.15 mm) in Kuthukadu, 35.0-97.2 mm (mean 61.07 mm) in Pulikat lake and 34.1-99.0 mm (mean 71.97 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between greatest depth and all the characters studied for pooled sexes in different localities (Tables 3-10).

#### Dorso-anal depth ( $D_1A_1$ )

Differences were significant in respect of dorso-anal depth in the samples of different sexes as well as pooled out sexes in different localities. Mean dorso-anal depth was recorded as 60.06 mm (range 26.5-114.5 mm) in Cochin, 61.76 mm (range 29.1-93.5 mm) in Mangalore, 53.48 mm (range 35.2-68.0 mm)

in Karwar, 54.15 mm (range 23.8-65.7 mm) in Goa, 57.24 mm (range 27.2-71.5 mm) in Pondicherry, 58.14 mm (range 33.0-79.00 mm) in Muthukadu, 59.82 mm (range 33.5-96.2 mm) in Pulikat lake and 70.75 mm (33.1-96.0 mm) in Hyderabad (Table 2).

Correlations were positive and significant between dorso-anal depth and all the characters studied in different localities (Tables 3-10).

#### Perpendicular anal depth ( $A_1A_2$ )

Perpendicular anal depth showed significant differences between different sexes as well as in sex pooled samples in different localities (Table 1 and 2). The range recorded for perpendicular anal depth of pooled sample was 26.5-110.9mm (mean 60.25 mm) in Cochin, 29.6-93.0 mm (mean 62.16 mm) in Mangalore, 35.7-67.0 mm (mean 53.65 mm) in Karwar, 24.2-66.5 mm (mean 54.54 mm) in Goa, 27.9-69.5 mm (mean 57.23 mm) in Pondicherry, 32.8-79.5 mm (mean 57.95 mm) in Muthukadu, 34.2-95.0 mm (mean 60.36 mm) in Pulikat lake and 33.5-98.5 mm (mean 71.08 mm) in Hyderabad. (Table 2).

Positive and significant correlations were observed between perpendicular anal depth and all the characters of sex pooled samples in different localities. (Tables 3-10).

#### Caudal peduncle depth (CPD)

Differences were significant in mean caudal peduncle depth in different sexes as well as pooled out sex in different

localities (Tables 1 and 2). Mean caudal peduncle depth varied between 7.5-42.4 mm (mean 19.39 mm) in Cochin, 8.5-29.0 mm (mean 18.51 mm) in Mangalore, 10.1-23.0 mm (mean 16.08 mm) in Karwar, 6.22-20.4 mm (mean 16.19 mm) in Goa, 7.2-22.0mm (mean 16.93 mm) in Pondicherry, 9.5-27.1 mm (mean 18.27 mm) in Muthukadu, 9.8-31.2 mm (mean 18.57 mm) in Pulikat lake and 9.5-29.2 mm (mean 20.44 mm) in Hyderabad (Table 2).

Correlations between caudal peduncle depth and all the characters in sex pooled samples of different localities were positive and significant (Tables 3-10).

#### Snout width across pre-orbital process (POW)

Snout width across pre-orbital process showed significant differences in different sexes in sex pooled samples in different localities. The range observed for POW of sex pooled samples was 5.5-33.5 mm (mean 13.11 mm) in Cochin, 5.8-24.2 mm (mean 13.55 mm) in Mangalore, 6.8-17.1 mm (mean 11.32 mm) in Karwar, 4.5-16.0mm (mean 11.30 mm) in Goa, 5.2-17.8 mm (mean 12.97 mm) in Pondicherry, 6.5-18.8 mm (mean 12.90 mm) in Muthukadu, 6.5-22.5 mm (mean 12.77 mm) in Pulikat lake and 7.0-24.2 mm (mean 16.21 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between POW and all the characters studied in different localities (Tables 3-10).

### Snout width across lachrymal (LAW)

There were significant differences in respect of snout width across lachrymal (LAW) in different sexes as well as in sex pooled samples in different localities (Tables 1 and 2). The range recorded for LAW in sex pooled samples was 3.7-22.5 mm (mean 8.93 mm) in Cochin, 4.2-15.5 mm (mean 8.86 mm) in Mangalore, 4.5-13.5 mm (mean 8.21 mm) in Karwar, 3.0-11.8 mm (mean 8.28 mm) in Goa, 3.8-17.2 mm (mean 9.00 mm) in Pondicherry, 4.0-13.2 mm (mean 9.12 mm) in Muthukadu, 5.7-17.8 mm (mean 10.28 mm) in Pulikat lake, and 5.9-20.0 mm (mean 12.54 mm) in Hyderabad (Table 2).

Correlations observed between LAW and all the characters of sex pooled samples were positive and significant in different localities (Tables 3-10).

### Width across pectoral fin (PP)

Differences were significant in respect of width across pectoral fin in different sexes and sex pooled samples of different localities (Table 1 and 2). Width across pectoral fin varied between 7.6-41.5 mm (mean 22.40 mm) in Cochin, 8.8-31.5 mm (mean 16.63 mm) in Mangalore, 12.0-23.5 mm (mean 16.89 mm) in Karwar, 6.1-19.5 mm (mean 16.45 mm) in Goa, 5.6-28.6 mm (mean 19.01 mm) in Pondicherry, 11.0-31.8 mm (mean 20.03 mm) in Muthukadu, 9.7-30.5 mm (mean 18.14 mm) in Pulikat lake and 12.7-31.2 mm (mean 22.12 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between width across pectoral fin and all the characters of sex pooled samples in different localities (Table 3-10).

#### Greatest width (GB)

There were significant differences in respect of greatest width between sexes and pooled out sexes in different localities. Mean greatest width recorded in sex pooled samples was 28.02 mm (range 7.9-55.5 mm) in Cochin, 20.44 mm (10.5-33.8 mm) in Mangalore, 18.73 mm (range 12.5-31.2 mm) in Karwar, 18.83 mm (range 6.8-23.2 mm) in Goa, 20.43 mm (range 7.3-29.9 mm) in Pondicherry, 23.19 mm (range 12.0-50.3 mm) in Muthukadu, 20.32 mm (range 10.3-31.7 mm) in Pulikat lake and 23.97 mm (range 13.5-34.0 mm) in Hyderabad (Table-2).

Correlations were positive and significant between greatest width of sex pooled sample and all the characters in different localities (Tables 3-10).

#### Inter-orbital width (IOW)

Differences were significant in respect of inter orbital width in different sexes and in pooled out sexes of different localities (Tables 1 and 2). The range of inter orbital width varied between 5.0-30.8 mm (mean 13.11 mm) in Cochin, 6.5-22.0 mm (mean 13.33 mm) in Mangalore, 6.5-17.1 mm (mean 11.29 mm) in Karwar, 5.0-15.0 mm (mean 11.15 mm) in Goa, 3.5-16.0 mm (mean 12.87 mm) in Pondicherry, 6.2-17.3 mm (mean 12.72 mm) in Muthukadu, 7.5-22.0 mm (mean 13.25 mm) in Pulikat lake, and 7.0-23.0 mm (mean 15.54 mm) in Hyderabad (Table 2).



Positive and significant correlations were observed between inter orbital width and all the characters of sex pooled samples in different localities (Tables 3-10).

#### Caudal peduncle width (CPE)

There was significant difference in respect of caudal peduncle width between sexes as well as in sex pooled samples of different localities (Tables 1 and 2). The range of caudal peduncle width of pooled sexes varied between 1.5-15.5 mm (mean 5.75 mm) in Cochin, 2.0-8.8 mm (mean 5.40 mm) in Mangalore, 2.6-7.3 mm (mean 4.38 mm) in Karwar, 1.8-6.7 mm (mean 4.45 mm) in Goa, 2.0-7.2 mm (mean 5.34 mm) in Pondicherry, 2.0-9.1 mm (mean 5.15 mm) in Muthukadu, 3.5-8.18 mm (mean 6.82 mm) in Pulikat lake and 2.8-10.8 (mean 6.60 mm) in Hyderabad (Table 2).

Correlations between caudal peduncle width and all the characters were positive and significant in pooled out sexes of different localities (Tables 3-10).

#### Lower jaw width (LJW)

Significant differences were observed in respect of lower jaw width in different sexes as well as in sex pooled samples of different localities (Table 1 & 2). The range recorded for lower jaw width of sex pooled sample was 4.0-22.5 mm (mean 9.09 mm) in Cochin, 4.5-15.0 mm (mean 5.91 mm) in Mangalore, 4.3-9.6 mm (mean 7.37 mm) in Karwar, 3.3-10.6 mm

(mean 7.38 mm) in Goa, 5.5-10.5 mm (mean 8.70 mm) in Pondicherry, 4.1-11.0 mm (mean 8.47 mm) in Muthukadu, 5.9-15.3 mm (mean 9.23 mm) in Pulikat lake, and 4.9-12.5 mm (mean 10.39 mm) in Hyderabad (Table-2).

Correlations were positive and significant between lower jaw width and all the characters studied in sex pooled samples of different localities (Tables 3-10).

#### Length of pectoral fin (PH)

Differences were significant in respect of length of pectoral fin in different sexes as well as in the sex pooled samples of different localities. (Tables 1 and 2). Mean length of pectoral fin recorded as 30.71 mm (range 13.5-59.0 mm) in Cochin 30.34 mm (range 15.2-46.5 mm) in Mangalore, 26.66 mm (range 17.8-37.7 mm) in Karwar, 26.56 mm (range 11.5-33.2 mm) in Goa, 28.79 mm (range 13.6-34.8 mm) in Pondicherry, 29.86 mm (range 17.5-41.2 mm) in Muthukadu, 29.98 mm (range 17.0-47.5 mm) in Pulikat lake and 33.88 mm (range 17.1-48.9 mm) in Hyderabad (Table 2).

Positive and significant correlations were observed between length of pectoral fin and all the characters of sex pooled samples in different localities (Tables 3-10).

#### Length of ventral fin (VH)

There was significant difference in respect of length of ventral fin between sexes and in pooled out sexes of

different localities (Table 1 and 2). The range for length of ventral fin in the sex pooled samples varied between 10.2-46.7 mm (mean 23.55 mm) in Cochin, 11.8-36.2 mm (mean 23.72 mm) in Mangalore, 12.9-30.8 mm (mean 21.34 mm) in Karwar, 10.0-21.2 mm (mean 21.65 mm) in Goa, 9.7-25.1 mm (mean 21.16 mm) in Pondicherry, 12.5-33.3 mm (mean 23.59 mm) in Muthukadu, 13.8-36.1 mm (mean 24.00 mm) in Pulikat lake and 12.5-32.0 mm (mean 23.98 mm) in Hyderabad (Table 2).

Correlations between length of ventral fin and all the characters were positive and significant for sex pooled samples in different localities (Tables 3-10).

#### Longest dorsal fin ray (LDFR)

Differences were significant in respect of longest dorsal fin ray in different sexes as well as in pooled out sexes of different localities (Table 1 and 2). Mean longest dorsal fin ray in sex pooled samples was recorded as 22.81 mm (range 6.5-63.6 mm) in Cochin, 22.32 mm (range 9.2-41.0 mm) in Mangalore, 19.30 mm (range 9.0-33.1 mm) in Karwar, 18.45 mm (range 5.0-29.0 mm) in Goa, 21.50 mm (range 7.0-28.5 mm) in Pondicherry, 24.86 mm (range 8.0-42.5 mm) in Muthukadu, 22.44 mm (range 8.2-45.0 mm) in Pulikat lake and 29.23 mm (range 9.3-45.8 mm) in Hyderabad (Tables 2).

There were positive and significant correlations between largest dorsal fin ray and all the characters in pooled out sexes of different localities (Tables 3-10).

### Longest anal fin ray (LAFR)

There was significant difference in respect of longest anal fin ray between sexes as well as in pooled out sexes in different localities (Tables 1 and 2). The range of longest anal fin ray varied in sex pooled samples between 6.3-49.5 mm (mean 20.84 mm) in Cochin, 9.2-39.0 mm (mean 21.73 mm) in Mangalore, 9.0-28.3 mm (mean 18.30 mm) in Karwar, 4.9-27.1 mm (mean 17.62 mm) in Goa, 6.5-27.2 mm (mean 21.03 mm) in Pondicherry, 8.0-42.5 mm (mean 22.94 mm) in Muthukadu, 8.2-45.0 mm (mean 21.39 mm) in Pulikat lake and 8.7-40.6 mm (Mean 26.89mm) in Hyderabad (Table 2).

Correlations between longest anal fin ray and all the characters of sex pooled samples studied in different localities were positive and significant (Tables 3-10).

Correlations between all morphometric characters studied were significant in different localities for pooled out sexes (Tables 3-10). Nine characters which represented linear, vertical and lateral measurements were selected for further statistical analysis to establish the variation between the localities in respect of these characters such as weight, standard length, lower jaw length, caudal peduncle length, eye diameter (vertical), greatest depth, width across pectoral fin, interorbital width and longest dorsal fin ray. Statistical method used was analysis of dispersion as described by Rao (1974).

TABLE - 11 DETAILS OF DORSAL FIN RAYS BRANCHED (DBR) AND DORSAL FIN RAYS SPINOUS (DPSR) OF TWO SPECIES OF ETROPLUS COLLECTED FROM DIFFERENT LOCALITIES OF PERINULAR INDIA

Sl. No.	Species	Locality	Size range (mm)	No. of specimens	Range of character	Mean	St. deviation	St. error of mean
<u>DORSAL FIN RAYS BRANCHED (DBR)</u>								
1	<u>ETROPLUS</u> <u>TERMINATUS</u>	COCHIN	25.2-310.0	52	14-17	15.03	0.70	0.09
		MANGALORE	67.0-217.0	76	13-16	14.77	0.73	0.08
		KARWAR	75.0-172.0	83	13-15	14.27	0.64	0.07
		GOA	53.0-155.0	95	13-16	14.65	0.67	0.06
		PONDICHERRY	63.0-170.0	58	14-15	14.41	0.49	0.06
		MUTHUKADU	74.2-210.0	62	13-15	14.08	0.74	0.09
		PULIKAT LAKE	67.5-225.0	58	14-16	14.98	0.75	0.09
		HYDERABAD	75.0-212.0	19	14-15	14.73	0.44	0.10
2	<u>ETROPLUS</u> <u>VAGILATUS</u>	COCHIN	53.0-90.0	63	8-11	9.52	0.73	0.08
		MUTHUKADU	45.0-91.0	64	8-10	9.32	0.70	0.08
		PULIKAT LAKE	48.0-98.0	60	8-11	9.36	1.06	0.13
		HYDERABAD	48.0-91.0	60	8-10	8.35	0.54	0.06

Sl. No.	SPECIES	Locality	Size range (mm)	No. of specimens	Range of character	Mean	St. deviation	St. error of mean
1	<u>ETROPLUS SURATENSIS</u>		DORSAL FIN RAYS	SPINOUS (DFSP)				
		COCHIN	25.2-310.0	52	17-19	18.00	0.43	0.05
		MANGALORE	67.0-217.0	76	17-19	18.18	0.50	0.05
		KARWAR	75.0-172.0	83	18-19	18.60	0.48	0.05
		GOA	53.0-155.0	95	15-19	17.89	0.49	0.05
		PONDICHERRY	63.0-170.0	58	17-19	18.67	0.50	0.06
		MUTHUKADU	74.2-210.0	62	17-19	18.09	0.75	0.09
		PULIKAT LAKE	67.5-225.0	58	18-19	18.50	0.50	0.06
		HYDERABAD	75.0-212.0	19	18-19	18.15	0.36	0.08
2	<u>ETROPLUS MACULATUS</u>	COCHIN	53.0-90.0	83	17-19	18.04	0.64	0.07
		MUTHUKADU	45.0-91.0	64	17-18	17.53	0.49	0.06
		PULIKAT LAKE	48.0-98.0	60	18-19	18.41	0.49	0.06
		HYDERABAD	48.0-91.0	60	17-19	17.51	0.53	0.06

TABLE - 12 DETAILS OF ANAL FIN RAYS BRANCHED (AFBR) AND DORSAL FIN RAYS SPINOUS (AFSP) OF TWO SPECIES OF ETROPIUS COLLECTED FROM DIFFERENT LOCALITIES OF PENINSULAR INDIA

Sl. No.	Species	Locality	Size range (mm)	No. of specimens	Range of character	Mean	St. deviation	St. error of mean
ANAL FIN RAYS BRANCHED (AFBR)								
1.	<u>ETROPIUS</u> <u>MACULATUS</u>	COCHIN	25.2-310.0	52	11-13	12.34	0.55	0.07
		MANGALORE	67.0-172.0	76	11-13	12.06	0.49	0.05
		KARWAR	75.0-172.0	83	10-13	11.59	0.65	0.07
		GOA	53.0-155.0	94	11-13	11.82	0.56	0.05
		PONDICHERRY	63.0-170.0	58	11-13	11.79	0.54	0.07
		MUTHUKADU	74.2-210.0	62	11-13	11.74	0.82	0.10
		PULIKAT LAKE	67.5-225.0	58	11-13	12.06	0.71	0.09
		HYDERABAD	75.0-212.0	19	11-13	11.94	0.88	0.20
2.	<u>ETROPIUS</u> <u>MACULATUS</u>	COCHIN	53.0- 90.0	83	7-10	8.67	0.66	0.07
		MUTHUKADU	45.0- 91.0	64	7-10	8.21	0.89	0.11
		PULIKAT LAKE	48.0-98.0	60	7-8	7.38	0.48	0.06
		HYDERABAD	48.0-91.0	60	7-8	7.56	0.52	0.06

Sl. No.	Species	Locality	Size range (mm)	No. of speci- mens	Range of character	Mean	St. deviation	St. error of mean
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ANAL FIN RAYS SPINOUS (AFSP)

1.	<u>ETROPLUS SUKATENSIS</u>	COCHIN	25.2-310.0	52	11-13	12.09	0.52	0.07
		MANGALORE	67.0-217.0	76	11-13	12.14	0.50	0.05
		KARWAR	75.0-172.0	83	11-14	12.44	0.60	0.06
		GOA	53.0-155.0	95	10-13	11.74	0.61	0.06
		PONDICHERRY	63.0-170.0	58	10-13	12.48	0.62	0.08
		MUTHUKADU	74.2-210.0	62	10-14	11.61	0.65	0.08
		FULIKAT LAKE	67.5-225.0	58	11-13	11.94	0.68	0.08
		HYDERABAD	75.0-212.0	19	11-13	11.89	0.71	0.16
2.	<u>ETROPLUS MACULATUS</u>	COCHIN	53.0-90.0	83	12-14	12.92	0.59	0.06
		MUTHUKADU	45.0-91.0	64	11-13	12.17	0.65	0.10
		FULIKAT LAKE	48.0-98.0	60	12-14	13.10	0.70	0.09
		HYDERABAD	48.0-91.0	60	12-13	12.73	0.44	0.05



Sl. No.	Species	Locality	Size range (mm)	No. of specimens	Range of character	Mean	St. deviation	St. error of mean
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VERTEBRAE CAUDAL (VC)

1	<u>ETROPLUS</u> <u>S. AD.</u> <u>SIS</u>	COCHIN	25.2-310.0	52	10-15	13.90	0.62	0.08
		MANGALORE	67.0-217.0	76	12-15	14.00	0.56	0.06
		KARWAR	75.0-172.0	83	13-15	14.08	0.31	0.03
		GOA	53.0-155.0	95	13-15	13.93	0.45	0.04
		PONDICHERRY	63.0-170.0	58	14-15	14.50	0.50	0.06
		MUTHUKALU	74.2-210.0	62	13-15	13.91	0.74	0.09
		PULIKAT LAKE	67.5-225.0	58	14-15	13.39	0.48	0.06
		HYDERABAD	75.0-212.0	19	14-15	14.42	0.49	0.11
2	<u>ETROPLUS</u> <u>MACHILATUS</u>	COCHIN	53.0-90.0	83	12-14	13.85	0.38	0.04
		MUTHUKALU	45.0-91.0	64	12-15	13.57	0.96	0.12
		PULIKAT LAKE	48.0-98.0	60	13-14	13.51	0.49	0.06
		HYDERABAD	48.0-91.0	60	12-14	13.10	0.70	0.09

TABLE 14 DETAILS OF GILL RAKERS UPPER (GRU) AND LOWER (GL) OF TWO SPECIES OF ETROPLUS COLLECTED FROM DIFFERENT LOCALITIES OF PENINSULAR INDIA

Sl. No.	Species	Locality	Size of range (mm)	No. of specimens	Range of character	Mean	St. deviation	St. error of mean
1	<u>ETROPLUS SURATENSIS</u>		GILL RAKERS UPPER (GRU)					
		COCHIN	25.2-310.0	52	7-9	8.34	0.58	0.08
		MANGALORE	67.0-217.0	76	8-9	8.01	0.11	0.01
		KARNAR	75.0-172.0	83	8-9	8.59	0.49	0.05
		GOA	53.0-155.0	95	7-9	7.93	0.53	0.05
		PONDICHERRY	63.0-170.0	58	8-9	8.32	0.56	0.07
		MUTHUKADU	74.2-210.0	62	8-9	8.51	0.49	0.06
		PULIKAT LAKE	67.5-225.0	58	7-9	8.03	0.99	0.12
		HYDERABAD	75.0-212.0	19	8-9	8.52	0.49	0.11
2	<u>ETROPLUS MACULATUS</u>	COCHIN	53.0-90.0	83	5-8	6.95	0.61	0.06
		MUTHUKADU	45.0-91.0	64	5-7	6.00	0.70	0.08
		PULIKAT LAKE	48.0-98.0	60	6-8	6.91	0.69	0.08
		HYDERABAD	48.0-98.0	60	5-8	6.11	0.87	0.11

Sl. No.	Species	Locality	Size range (mm)	No. of specimens	Range of character	Mean	St. deviation	St. Error of mean
1	<u>ETROPLUS SURATENSIS</u>		<u>GILL RAKERS LOWER (GRL)</u>					
		COCHIN	25.2-310.0	52	10-14	11.78	0.92	0.12
		MANGALORE	67.0-217.0	76	11-12	11.94	0.22	0.02
		KARNAR	75.0-172.0	83	10-12	11.60	0.57	0.06
		GOA	53.0-155.0	95	11-12	11.65	0.47	0.04
		PONDICHERRY	63.0-170.0	58	9-12	10.72	0.51	0.06
		MUTHUKADU	74.2-210.0	62	10-12	11.06	0.78	0.09
		PULIKAT LAKE	67.5-225.0	58	11-12	11.37	0.48	0.06
		HYDERABAD	75.0-212.0	19	11	11.00	0.00	0.00
2	<u>ETROPLUS MACULATUS</u>	COCHIN	53.0-90.0	83	9-10	9.80	0.39	0.04
		MUTHUKADU	45.0-91.0	64	9-11	9.92	0.66	0.08
		PULIKAT LAKE	48.0-98.0	60	8-10	9.11	0.70	0.09
		HYDERABAD	48.0-91.0	60	9-11	9.95	0.66	0.08

TABLE - 15 DETAILS OF GILL TEETH(GT) OF TWO SPECIES OF ETROPLUS COLLECTED FROM DIFFERENT LOCALITIES OF PENINSULAR INDIA.

Sl. No.	SPECIES	LOCALITY	Size of range	No. of specimens	Range of characters	Mean	St. deviation	St. error of mean
GILL TEETH (GT)								
1	<u>ETROPLUS</u> <u>SURATENSIS</u>	COCHIN	25.2-310.0	52	16-19	17.05	0.94	0.13
		MANGALORE	67.0-217.0	76	14-18	17.85	0.72	0.08
		KANNAR	75.0-172.0	83	14-18	17.90	0.52	0.05
		GCA	53.0-155.0	95	16-18	17.62	0.50	0.05
		PON. ICHERRY	63.0-170.0	58	16-18	17.31	0.77	0.10
		MUTHUKADU	74.2-210.0	62	16-17	16.53	0.49	0.06
		FULIKAT LAKE	67.5-225.0	58	15-18	16.37	0.94	0.12
		HYDERABAD	75.0-212.0	19	17-18	17.36	0.48	0.11
2	<u>ETROPLUS</u> <u>MACULATUS</u>	COCHIN	53.0-90.0	83	13-15	14.09	0.33	0.03
		MUTHUKADU	45.0-91.0	64	12-14	13.01	0.78	0.09
		FULIKAT LAKE	48.0-98.0	60	13-15	13.90	0.67	0.08
		HYDERABAD	48.0-91.0	60	13-15	14.05	0.69	0.08

TABLE - 16 SUMMARY OF THE RESULTS OF  $\chi^2$  TEST IN RESPECT OF MERISTIC COUNTS OF  
ETROPLUS SURATENSIS BETWEEN DIFFERENT LOCALITIES

Sl. No.	Localities	Degree of Freedom	CHARACTERS								
			DPBR	DPST	APBR	APSP	VA	VC	GRU	ORL	GT
1	Cochin Vs. Mangalore	126	**	**	**	NS	NS	NS	**	NS	**
2	Cochin Vs. Karwar	133	**	**	**	**	NS	**	**	NS	**
3	Cochin Vs. Goa	145	**	NS	**	**	NS	NS	**	NS	**
4	Cochin Vs. Pondicherry	100	**	**	**	**	**	**	NS	NS	NS
5	Cochin Vs. Muthukadu	112	**	NS	**	**	**	NS	NS	**	**
6	Cochin Vs. Pulikat lake	100	NS	**	**	NS	**	**	NS	**	**
7	Cochin Vs. Hyderabad	69	NS	NS	**	NS	NS	**	NS	**	NS
8	Mangalore Vs. Karwar	157	**	**	**	**	NS	**	**	**	NS
9	Mangalore Vs. Goa	167	NS	**	**	**	**	NS	NS	**	**
10	Mangalore Vs. Pondicherry	132	**	**	**	**	NS	**	**	**	**
11	Mangalore Vs. Muthukadu	136	**	NS	**	**	NS	NS	**	**	**
12	Mangalore vs. Pulikat lake	133	NS	**	NS	**	NS	**	NS	**	**
13	Mangalore Vs. Hyderabad	93	NS	NS	NS	NS	**	**	**	**	**

Sl. No.	Localities	Degree of Freedom	CHARACTERS								
			DFER	FDP	AFRR	APSP	VA	VC	TRU	GRL	GT
14	Karwar Vs. Goa	175	**	**	NS	**	**	**	**	NS	**
15	Karwar Vs. Pondicherry	139	NS	NS	NS	NS	NS	**	**	NS	**
16	Karwar Vs. Muthukadu	143	**	**	NS	**	NS	NS	NS	**	**
17	Karwar Vs. Pulikat lake	139	**	NS	**	**	**	**	**	**	**
18	Karwar Vs. Hyderabad	100	**	**	NS	**	**	**	NS	**	**
19	Goa Vs. Pondicherry	151	**	**	NS	**	**	NS	**	**	**
20	Goa Vs. Muthukadu	155	**	**	NS	NS	NS	NS	**	**	**
21	Goa Vs. Pulikat Lake	151	**	**	**	NS	**	**	NS	**	**
22	Goa Vs. Hyderabad	112	NS	**	NS	NS	NS	**	**	**	**
23	Pondicherry Vs. Muthukadu	118	**	**	NS	**	NS	**	**	**	**
24	Pondicherry Vs. Pulikat Lake	114	**	NS	**	**	NS	NS	NS	**	**
25	Pondicherry Vs. Hyderabad	75	**	**	NS	**	**	NS	NS	**	NS
26	Muthukadu Vs. Pulikat Lake	118	**	**	**	**	NS	**	**	**	NS
27	Muthukadu Vs. Hyderabad	79	**	NS	NS	NS	NS	**	NS	NS	**
28	Pulikat Vs. Hyderabad	75	NS	**	**	NS	NS	NS	**	**	**

Hyderabad, Mangalore-Goa, Mangalore-Pulikat lake, Mangalore-Hyderabad, Karwar-Pondicherry, Goa-Hyderabad and Pulikat lake-Hyderabad. Comparison of samples between these pairs of localities did not reveal significant difference ( $P > 0.05$ ) (Table 22).

#### Dorsal fin-spinous rays (DFSP)

Comparison of samples between Cochin-Goa, Cochin-Muthukadu, Cochin-Hyderabad, Mangalore-Muthukadu, Mangalore-Hyderabad, Karwar-Pondicherry, Karwar-Pulikat lake, Pondicherry-Pulikat lake and Muthukadu-Hyderabad revealed no significant differences ( $P > 0.05$ ), whereas significant ( $P < 0.05$ ) difference was observed between all other pairs of localities (Table 22).

#### Anal fin-branched rays (AFBR)

Comparison between samples of Mangalore-Pulikat lake, Mangalore-Hyderabad, Karwar-Goa, Karwar-Pondicherry, Karwar-Muthukadu, Karwar-Hyderabad, Goa-Pondicherry, Goa-Muthukadu, Goa-Hyderabad, Pondicherry-Muthukadu, Pondicherry-Hyderabad and Muthukadu-Hyderabad did not reveal any significant ( $P > 0.05$ ) difference, while between the other pairs of localities the differences were significant ( $P < 0.05$ ) (Table 22).

#### Anan fin spinous rays (AFSP)

Significant differences were not observed between samples of Cochin-Mangalore, Cochin-Pulikat lake, Cochin-

Hyderabad, Mangalore-Hyderabad, Karwar-Pondicherry, Goa-Muthukadu, Goa-Pulikat lake, Goa-Hyderabad, Muthukadu-Hyderabad and Pulikat-Hyderabad, while between other pairs of places the variations in the mean values showed significant difference ( $P < 0.05$ ) (Table 16).

#### Vertebrae-abdominal (VA)

Significant ( $P < 0.05$ ) differences were observed between samples from Cochin-Pondicherry, Cochin-Muthukadu, Cochin-Pulikat lake, Mangalore-Goa, Mangalore-Hyderabad, Karwar-Goa, Karwar-Pulikat lake, Karwar-Hyderabad, Goa-Pondicherry, Goa-Pulikat lake, and Pondicherry-Hyderabad. The difference in mean values between all other pairs of places were not significant ( $P > 0.05$ , Table 16).

#### Vertebrae-caudal (VC)

Comparison of samples of Cochin-Mangalore, Cochin-Goa, Cochin-Muthukadu, Mangalore-Goa, Mangalore-Muthukadu, Karwar-Muthukadu, Goa-Pondicherry, Goa-Muthukadu, Pondicherry-Pulikat lake, Pondicherry-Hyderabad and Pulikat lake-Hyderabad revealed no significant ( $P > 0.05$ ) difference, while between all other pairs of places the difference in mean values were significant ( $P < 0.05$ , Table 16).

#### Gill rakers-upper (GRU)

Cochin sample, when compared with that of Pondicherry, Muthukadu, Pulikat lake and Hyderabad did not show any signi-



significant difference. Similarly the samples of Mangalore-Coa, Mangalore-Pulikat lake, Karwar-Muthukadu, Karwar-Hyderabad, Coa-Pulikat lake, Pondicherry-Pulikat lake, Pondicherry-Hyderabad and Muthukadu-Hyderabad did not reveal any significant difference, while the difference between all other pairs of places were significant ( $P < 0.05$ , Table 16).

#### Gill rakers-lower (GR<sub>1</sub>)

Significant difference was not observed between samples from Cochin and that of Mangalore, Karwar, Coa and Pondicherry; between Karwar and that of Coa and Pondicherry, and between Muthukadu and Hyderabad, while the differences in mean values were significant ( $P < 0.05$ ) between all other pairs of places (Table 16).

#### Gill teeth (GT)

Comparison of samples between Cochin-Pondicherry, Cochin-Hyderabad, Mangalore-Karwar, Pondicherry-Hyderabad and Muthukadu-Pulikat lake revealed no significant difference, whereas between all other pairs of places the mean values were significant ( $P < 0.05$ , Table 16).

The results of 't' test applied to the mean values of meristic counts of samples of different localities are summarized in the table 16. It can be seen that significant differences were observed in more than four meristic characters between all the places, except between Cochin and Hyderabad,

Karwar and Pondicherry and Muthukadu and Hyderabad, where only three out of nine characters studied were significant ( $P < 0.05$ ).

Maximum of eight characters were significant between Mangalore-Pondicherry and Karwar-Pulikat lake; seven characters between Cochin-Karwar, Karwar-Goa, Karwar-Hyderabad, Goa-Pondicherry, Goa-Pulikat lake, Pondicherry-Muthukadu and Muthukadu-Pulikat lake; six characters between Cochin-Pondicherry, Cochin-Muthukadu, Cochin-Pulikat lake, Mangalore-Karwar, Mangalore-Goa and Mangalore-Muthukadu and five characters between Cochin-Mangalore, Cochin-Goa, Mangalore-Pulikat lake, Mangalore-Hyderabad, Karwar-Muthukadu, Goa-Muthukadu, Goa-Hyderabad, Pondicherry-Pulikat lake, Pondicherry-Hyderabad and Pulikat lake-Hyderabad.

From the above it is concluded that the population of Cochin-Hyderabad, Karwar-Pondicherry and Muthukadu-Hyderabad are probably derived from a single or closely related stocks, since the differences were not significant in respect of maximum six characters between them, whereas, all other populations showed significant differences in respect of five or more characters and thus can be considered as heterogeneous.

#### 4.5. Results of studies on morphometric characters of Etrorplus maculatus

##### Body weight (WT)

There were no significant differences in respect of the body weight between sexes (except in samples from Hyderabad) and in pooled out sexes in different localities (Table 17 and 18). The range recorded for body weight varied between 2.3-15.5 gm (mean 7.23 gm) in Cochin, 2.0-14.7 gm (mean 6.19 gm) in Muthukadu, 2.0-19.1 gm (mean 8.05 gm) in Pulikat lake and 2.4-14.6 gm (mean 6.46 gm) in Hyderabad (Table 18).

Correlations between body weight and all characters studied in pooled out sexes were positive and significant in different localities (Tables 19 to 22).

##### Total length (TL)

Differences were significant in respect of total length in pooled out sexes among all localities and between the sexes it was significant only between sexes in Pulikat lake and Hyderabad samples. Mean total length recorded in sex pooled samples was 70.41 mm (range 53.0-90.0 mm) in Cochin, 66.00 mm (range 45.0-91.0 mm) in Muthukadu, 72.07 mm (range 48.0-98.0 mm) in Pulikat lake and 66.63 mm (range 48.0-91.0 mm) in Hyderabad (Table 18).

Total length showed positive and significant correlations with all the characters of pooled out sexes studied in different localities (Tables 19-22).

TABLE - 17 MEAN AND STANDARD DEVIATION OF DIFFERENT CHARACTERS BETWEEN SEXES OF  
ETROPLUS MACULATUS IN DIFFERENT LOCALITIES

Sl. No.	Character	Sex	LOCALITY			
			Cochin	Mathukadu	Pulikat lake	Hyderabad
1	WT	Male	6.04 ( $\pm 3.02$ )	5.99 ( $\pm 2.70$ )	8.06 ( $\pm 4.31$ )	7.42 ( $\pm 2.80$ )
2		Female	6.36 ( $\pm 3.23$ )	6.28 ( $\pm 2.90$ )	7.35 ( $\pm 4.22$ )	4.78 ( $\pm 2.38$ )
2	TL	Male	66.64 ( $\pm 8.69$ )	64.99 ( $\pm 10.84$ )	72.01 ( $\pm 11.34$ )	70.43 ( $\pm 10.33$ )
		Female	67.09 ( $\pm 8.95$ )	66.45 ( $\pm 11.20$ )	66.81 ( $\pm 11.84$ )	59.61 ( $\pm 9.73$ )
3	SL	Male	50.53 ( $\pm 6.25$ )	48.71 ( $\pm 8.04$ )	54.28 ( $\pm 8.18$ )	53.38 ( $\pm 8.61$ )
		Female	51.48 ( $\pm 6.59$ )	49.36 ( $\pm 8.15$ )	52.73 ( $\pm 8.49$ )	44.78 ( $\pm 7.22$ )
4	HL	Male	17.24 ( $\pm 1.91$ )	17.90 ( $\pm 3.06$ )	18.56 ( $\pm 2.54$ )	19.74 ( $\pm 2.69$ )
		Female	17.28 ( $\pm 2.04$ )	18.19 ( $\pm 2.94$ )	18.17 ( $\pm 2.62$ )	16.73 ( $\pm 2.71$ )
5	SNL	Male	5.99 ( $\pm 0.09$ )	6.33 ( $\pm 1.58$ )	6.28 ( $\pm 1.98$ )	7.85 ( $\pm 1.25$ )
		Female	5.91 ( $\pm 0.88$ )	7.12 ( $\pm 1.64$ )	6.04 ( $\pm 1.05$ )	6.62 ( $\pm 1.23$ )

Sl. No.	Character	Sex	LOCALITY			
			Cochin	Muthukadu	Pulakat lake	Hyderabad
6	LJL	Male	4.13 ( $\pm 0.62$ )	3.93 ( $\pm 0.61$ )	5.02 ( $\pm 1.21$ )	4.44 ( $\pm 0.67$ )
		Female	4.35 ( $\pm 0.88$ )	3.77 ( $\pm 0.61$ )	4.83 ( $\pm 1.38$ )	3.83 ( $\pm 0.74$ )
7	LD	Male	22.02 ( $\pm 2.77$ )	22.97 ( $\pm 3.92$ )	23.86 ( $\pm 3.10$ )	24.98 ( $\pm 3.85$ )
		Female	22.17 ( $\pm 2.94$ )	23.35 ( $\pm 4.04$ )	23.33 ( $\pm 3.29$ )	20.99 ( $\pm 3.39$ )
8	LP	Male	18.12 ( $\pm 2.43$ )	18.77 ( $\pm 2.77$ )	19.78 ( $\pm 3.06$ )	21.08 ( $\pm 3.13$ )
		Female	18.35 ( $\pm 2.21$ )	19.38 ( $\pm 2.93$ )	19.06 ( $\pm 3.12$ )	18.15 ( $\pm 2.63$ )
9	LVL	Male	20.80 ( $\pm 2.31$ )	20.93 ( $\pm 3.95$ )	22.63 ( $\pm 2.94$ )	23.30 ( $\pm 3.36$ )
		Female	21.01 ( $\pm 2.44$ )	21.71 ( $\pm 4.02$ )	21.96 ( $\pm 3.00$ )	19.98 ( $\pm 3.36$ )
10	LA	Male	25.93 ( $\pm 2.69$ )	27.25 ( $\pm 3.92$ )	28.93 ( $\pm 4.30$ )	28.99 ( $\pm 4.51$ )
		Female	26.67 ( $\pm 3.54$ )	27.70 ( $\pm 4.22$ )	28.41 ( $\pm 4.48$ )	25.20 ( $\pm 4.00$ )
11	CPL	Male	4.51 ( $\pm 0.78$ )	4.25 ( $\pm 0.81$ )	4.39 ( $\pm 0.76$ )	4.48 ( $\pm 0.81$ )
		Female	4.36 ( $\pm 0.58$ )	4.46 ( $\pm 0.84$ )	4.43 ( $\pm 0.77$ )	3.83 ( $\pm 0.75$ )

Sl. No.	Character	Sex	LOCALITY			
			Cochin	Muthukadu	Pulikat Lake	Hyderabad
12	EYL	Male	5.72 ( $\pm 0.67$ )	5.80 ( $\pm 1.18$ )	6.18 ( $\pm 1.02$ )	6.55 ( $\pm 0.80$ )
		Female	5.78 ( $\pm 0.71$ )	5.87 ( $\pm 1.12$ )	5.93 ( $\pm 1.19$ )	5.76 ( $\pm 0.80$ )
13	CFL	Male	16.46 ( $\pm 2.46$ )	16.13 ( $\pm 2.99$ )	17.13 ( $\pm 2.21$ )	17.35 ( $\pm 2.88$ )
		Female	16.29 ( $\pm 2.11$ )	16.54 ( $\pm 3.01$ )	16.82 ( $\pm 2.32$ )	14.24 ( $\pm 2.30$ )
14	EYD	Male	5.56 ( $\pm 0.64$ )	5.97 ( $\pm 0.90$ )	6.03 ( $\pm 1.04$ )	6.34 ( $\pm 0.78$ )
		Female	5.68 ( $\pm 0.68$ )	5.76 ( $\pm 0.90$ )	5.94 ( $\pm 1.07$ )	5.56 ( $\pm 0.83$ )
15	HD	Male	13.71 ( $\pm 1.55$ )	13.82 ( $\pm 2.69$ )	14.87 ( $\pm 2.49$ )	14.77 ( $\pm 2.43$ )
		Female	13.78 ( $\pm 1.93$ )	14.24 ( $\pm 2.80$ )	14.38 ( $\pm 2.49$ )	12.66 ( $\pm 2.43$ )
16	CHD	Male	4.71 ( $\pm 0.85$ )	5.97 ( $\pm 1.73$ )	5.35 ( $\pm 1.41$ )	6.45 ( $\pm 1.18$ )
		Female	4.79 ( $\pm 1.03$ )	6.11 ( $\pm 1.62$ )	5.01 ( $\pm 1.41$ )	5.26 ( $\pm 1.13$ )

Sl. No.	Character	Sex	LOCALITY			
			Cochin	Muthukadu	Palikat lake	Hyderabad
17	GH	Male	27.23 ( $\pm 3.41$ )	28.10 ( $\pm 4.07$ )	29.66 ( $\pm 3.66$ )	29.26 ( $\pm 3.89$ )
		Female	27.68 ( $\pm 3.71$ )	28.53 ( $\pm 4.78$ )	29.01 ( $\pm 3.74$ )	24.99 ( $\pm 3.78$ )
18	D <sub>1</sub> A <sub>1</sub>	Male	26.78 ( $\pm 3.35$ )	26.77 ( $\pm 4.32$ )	28.84 ( $\pm 3.83$ )	29.37 ( $\pm 4.33$ )
		Female	27.37 ( $\pm 3.56$ )	27.58 ( $\pm 4.65$ )	28.34 ( $\pm 3.66$ )	24.82 ( $\pm 3.93$ )
19	A <sub>1</sub> A <sub>2</sub>	Male	27.05 ( $\pm 3.34$ )	27.26 ( $\pm 4.34$ )	29.16 ( $\pm 3.61$ )	29.14 ( $\pm 4.08$ )
		Female	27.45 ( $\pm 3.55$ )	27.89 ( $\pm 4.82$ )	28.40 ( $\pm 3.56$ )	24.62 ( $\pm 3.75$ )
20	CPD	Male	7.90 ( $\pm 1.24$ )	8.20 ( $\pm 1.52$ )	8.65 ( $\pm 1.40$ )	8.98 ( $\pm 1.34$ )
		Female	8.07 ( $\pm 1.20$ )	8.53 ( $\pm 1.54$ )	8.26 ( $\pm 1.45$ )	7.54 ( $\pm 1.06$ )
21	PCW	Male	5.65 ( $\pm 0.76$ )	5.91 ( $\pm 1.13$ )	6.16 ( $\pm 1.00$ )	6.56 ( $\pm 1.17$ )
		Female	5.76 ( $\pm 0.86$ )	5.96 ( $\pm 1.17$ )	6.01 ( $\pm 1.02$ )	5.46 ( $\pm 1.06$ )

Sl. No.	Character	Sex	LOCALITY			
			Cochin	Muthukadu	Pulikat lake	Hyderabad
22	LAW	Male	4.42 ( $\pm 0.72$ )	4.89 ( $\pm 0.99$ )	4.79 ( $\pm 0.99$ )	5.28 ( $\pm 1.06$ )
		Female	4.41 ( $\pm 0.75$ )	5.03 ( $\pm 1.05$ )	4.64 ( $\pm 0.98$ )	4.33 ( $\pm 0.93$ )
23	PP	Male	8.56 ( $\pm 1.28$ )	8.37 ( $\pm 1.53$ )	9.75 ( $\pm 1.62$ )	9.12 ( $\pm 1.48$ )
		Female	9.15 ( $\pm 1.45$ )	8.56 ( $\pm 1.74$ )	9.41 ( $\pm 1.58$ )	7.93 ( $\pm 1.37$ )
24	GB	Male	9.23 ( $\pm 1.35$ )	8.77 ( $\pm 1.89$ )	10.08 ( $\pm 1.84$ )	10.15 ( $\pm 1.58$ )
		Female	9.86 ( $\pm 1.51$ )	8.01 ( $\pm 1.62$ )	9.71 ( $\pm 1.87$ )	8.74 ( $\pm 1.28$ )
25	IOW	Male	5.45 ( $\pm 0.89$ )	5.90 ( $\pm 1.14$ )	6.21 ( $\pm 1.32$ )	6.40 ( $\pm 1.10$ )
		Female	5.54 ( $\pm 0.88$ )	6.05 ( $\pm 1.03$ )	6.23 ( $\pm 1.23$ )	5.20 ( $\pm 0.94$ )
26	CPW	Male	2.60 ( $\pm 0.47$ )	2.60 ( $\pm 0.60$ )	3.03 ( $\pm 0.81$ )	3.00 ( $\pm 0.58$ )
		Female	2.63 ( $\pm 0.45$ )	2.67 ( $\pm 0.43$ )	2.88 ( $\pm 0.87$ )	2.51 ( $\pm 0.54$ )



Sl. No.	Character	Sex	LOCALITY			
			Cochin	Muthukadu	Pulikat lake	Hyderabad
27	LWN	Male	3.87 ( $\pm 0.68$ )	3.87 ( $\pm 0.85$ )	4.21 ( $\pm 0.71$ )	4.41 ( $\pm 0.77$ )
		Female	3.97 ( $\pm 0.69$ )	4.03 ( $\pm 0.85$ )	4.29 ( $\pm 0.74$ )	3.72 ( $\pm 0.73$ )
28	PH	Male	14.65 ( $\pm 1.81$ )	14.74 ( $\pm 2.28$ )	15.94 ( $\pm 2.41$ )	15.81 ( $\pm 2.29$ )
		Female	15.03 ( $\pm 1.96$ )	15.20 ( $\pm 2.37$ )	15.54 ( $\pm 2.33$ )	13.11 ( $\pm 2.50$ )
29	VH	Male	10.18 ( $\pm 1.18$ )	9.73 ( $\pm 1.54$ )	11.18 ( $\pm 1.77$ )	10.81 ( $\pm 1.45$ )
		Female	10.11 ( $\pm 1.20$ )	9.92 ( $\pm 1.33$ )	10.93 ( $\pm 1.79$ )	9.26 ( $\pm 1.34$ )
30	LDFR	Male	8.38 ( $\pm 1.30$ )	7.91 ( $\pm 1.85$ )	9.53 ( $\pm 1.66$ )	8.49 ( $\pm 1.57$ )
		Female	8.10 ( $\pm 1.14$ )	9.10 ( $\pm 2.05$ )	9.15 ( $\pm 1.61$ )	6.73 ( $\pm 1.19$ )
31	LAFR	Male	8.04 ( $\pm 1.19$ )	7.92 ( $\pm 1.91$ )	9.05 ( $\pm 1.62$ )	8.49 ( $\pm 1.47$ )
		Female	7.90 ( $\pm 1.07$ )	8.10 ( $\pm 2.04$ )	8.70 ( $\pm 1.66$ )	6.62 ( $\pm 1.41$ )

TABLE - 12 MEAN AND STANDARD DEVIATION OF DIFFERENT CHARACTERS IN SIX POOLED  
SAMPLES OF ETROPLUS MACULATUS IN DIFFERENT LOCALITIES

Sl. No.	Character	LOCALITY			
		Cochin	Muthukadu	Pulikat lake	Hyderabad
1	WT	7.23 ( $\pm 3.24$ )	6.19 ( $\pm 2.77$ )	8.05 ( $\pm 4.24$ )	6.46 ( $\pm 2.83$ )
2	TL	70.41 ( $\pm 8.44$ )	66.00 ( $\pm 10.85$ )	72.07 ( $\pm 11.30$ )	66.63 ( $\pm 10.77$ )
3	SL	53.44 ( $\pm 6.25$ )	49.24 ( $\pm 7.96$ )	54.37 ( $\pm 8.04$ )	50.32 ( $\pm 8.65$ )
4	HL	18.03 ( $\pm 1.92$ )	18.13 ( $\pm 2.94$ )	18.59 ( $\pm 2.54$ )	18.70 ( $\pm 2.87$ )
5	SNL	6.33 ( $\pm 0.93$ )	6.97 ( $\pm 1.60$ )	6.26 ( $\pm 0.99$ )	7.33 ( $\pm 1.37$ )
6	LJL	4.35 ( $\pm 0.85$ )	3.87 ( $\pm 0.59$ )	5.03 ( $\pm 1.27$ )	4.23 ( $\pm 0.73$ )
7	LD	23.10 ( $\pm 2.85$ )	23.28 ( $\pm 3.89$ )	23.97 ( $\pm 3.00$ )	23.58 ( $\pm 3.91$ )
8	LP	19.17 ( $\pm 2.01$ )	19.25 ( $\pm 2.80$ )	19.72 ( $\pm 3.03$ )	20.02 ( $\pm 3.11$ )
9	LVL	21.86 ( $\pm 2.24$ )	21.44 ( $\pm 3.92$ )	22.63 ( $\pm 2.86$ )	22.14 ( $\pm 3.52$ )
10	LA	27.42 ( $\pm 2.96$ )	27.60 ( $\pm 3.97$ )	29.16 ( $\pm 4.14$ )	27.70 ( $\pm 4.44$ )

Sl. No.	Character	LOCALITY			
		Cochin	Muthukadu	Palikat lake	Hyderabad
11	CPL	4.62 ( $\pm 0.76$ )	4.39 ( $\pm 0.81$ )	4.39 ( $\pm 0.78$ )	4.28 ( $\pm 0.79$ )
12	EYL	5.97 ( $\pm 0.70$ )	5.89 ( $\pm 1.12$ )	6.16 ( $\pm 1.06$ )	6.28 ( $\pm 0.84$ )
13	CPL	17.22 ( $\pm 2.38$ )	16.42 ( $\pm 2.95$ )	17.17 ( $\pm 2.23$ )	16.20 ( $\pm 2.94$ )
14	EYD	5.81 ( $\pm 0.70$ )	5.89 ( $\pm 0.91$ )	6.05 ( $\pm 1.06$ )	6.08 ( $\pm 0.85$ )
15	HD	14.38 ( $\pm 1.67$ )	14.12 ( $\pm 2.67$ )	14.83 ( $\pm 2.39$ )	14.08 ( $\pm 2.47$ )
16	CHD	5.09 ( $\pm 0.92$ )	6.09 ( $\pm 1.64$ )	5.30 ( $\pm 1.41$ )	6.02 ( $\pm 1.25$ )
17	GH	28.81 ( $\pm 3.41$ )	28.46 ( $\pm 4.32$ )	29.71 ( $\pm 3.58$ )	27.75 ( $\pm 4.12$ )
18	D <sub>1</sub> A <sub>1</sub>	28.41 ( $\pm 3.26$ )	27.31 ( $\pm 4.41$ )	28.94 ( $\pm 3.47$ )	27.77 ( $\pm 4.44$ )
19	A <sub>1</sub> A <sub>2</sub>	28.56 ( $\pm 3.23$ )	27.72 ( $\pm 4.48$ )	29.17 ( $\pm 3.47$ )	27.53 ( $\pm 4.25$ )
20	CPD	8.39 ( $\pm 1.27$ )	8.41 ( $\pm 1.50$ )	8.62 ( $\pm 1.37$ )	8.44 ( $\pm 1.38$ )

Sl. No.	Character	LOCALITY			
		Cochin	Muthukadu	Pulikat lake	Hyderabad
21	PCW	5.98 ( $\pm 0.31$ )	5.97 ( $\pm 1.11$ )	6.18 ( $\pm 0.93$ )	6.16 ( $\pm 1.20$ )
22	LAW	4.64 ( $\pm 0.78$ )	4.99 ( $\pm 1.00$ )	4.81 ( $\pm 0.96$ )	4.93 ( $\pm 1.00$ )
23	PP	9.29 ( $\pm 1.38$ )	8.51 ( $\pm 1.60$ )	9.74 ( $\pm 1.57$ )	8.73 ( $\pm 1.45$ )
24	GB	10.02 ( $\pm 1.39$ )	8.93 ( $\pm 1.74$ )	10.06 ( $\pm 1.83$ )	9.65 ( $\pm 1.54$ )
25	ICW	5.73 ( $\pm 0.97$ )	6.01 ( $\pm 1.06$ )	6.29 ( $\pm 1.28$ )	5.92 ( $\pm 1.19$ )
26	GPB	2.78 ( $\pm 0.44$ )	2.65 ( $\pm 0.52$ )	3.04 ( $\pm 0.81$ )	2.84 ( $\pm 0.58$ )
27	LJW	4.10 ( $\pm 0.72$ )	3.97 ( $\pm 0.85$ )	4.29 ( $\pm 0.72$ )	4.18 ( $\pm 0.81$ )
28	PH	15.42 ( $\pm 1.96$ )	15.03 ( $\pm 2.29$ )	16.02 ( $\pm 2.24$ )	14.80 ( $\pm 2.67$ )
29	VH	10.62 ( $\pm 0.11$ )	9.87 ( $\pm 1.41$ )	11.23 ( $\pm 1.72$ )	10.28 ( $\pm 1.47$ )
30	LDPR	8.65 ( $\pm 1.25$ )	8.09 ( $\pm 1.90$ )	9.50 ( $\pm 1.61$ )	7.81 ( $\pm 1.62$ )
31	LAFR	8.36 ( $\pm 1.15$ )	8.08 ( $\pm 1.91$ )	9.04 ( $\pm 1.58$ )	7.81 ( $\pm 1.65$ )

Table - 19

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Table - 20 CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF *ETROPLUS MACULATUS* IN MUTHUKADU

No	Character	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CPL	EYD	HD	CHD	GH	DIA1	A1A2	CPD	POW	LAW	PP	GB	LOW	CFB	LJW	PH	VH	LDPR	LAFR
1	TL	1																													
2	SL	0.989	1																												
3	HL	0.959	0.980	1																											
4	SNL	0.960	0.926	0.958	1																										
5	LJL	0.788	0.961	0.962	0.965	1																									
6	LD	0.796	0.775	0.758	0.723	0.610	1																								
7	LP	0.979	0.980	0.946	0.843	0.954	0.989	1																							
8	LVL	0.976	0.958	0.880	0.930	0.971	0.919	0.967	1																						
9	LA	0.965	0.890	0.928	0.973	0.835	0.988	0.953	0.971	1																					
10	CPL	0.925	0.908	0.933	0.907	0.953	0.913	0.938	0.940	0.939	1																				
11	EYL	0.763	0.810	0.805	0.864	0.530	0.835	0.840	0.921	0.908	0.885	1																			
12	CPL	0.963	0.915	0.928	0.947	0.933	0.936	0.937	0.908	0.914	0.857	0.920	1																		
13	EYD	0.910	0.971	0.961	0.978	0.984	0.985	0.945	0.926	0.881	0.961	0.929	0.993	1																	
14	HD	0.893	0.916	0.896	0.892	0.894	0.905	0.901	0.879	0.899	0.913	0.836	0.723	0.884	1																
15	CHD	0.956	0.967	0.978	0.973	0.946	0.927	0.893	0.958	0.953	0.816	0.660	0.888	0.955	0.933	1															
16	GH	0.955	0.960	0.958	0.915	0.923	0.892	0.930	0.933	0.852	0.675	0.922	0.930	0.778	0.957	0.969	1														
17	DIA1	0.967	0.992	0.944	0.922	0.879	0.968	0.932	0.786	0.623	0.877	0.947	0.851	0.965	0.968		1														
18	A1A2	0.993	0.946	0.924	0.883	0.967	0.932	0.804	0.633	0.897	0.951	0.855	0.968	0.972			1														
19	CPD	0.939	0.915	0.869	0.952	0.925	0.777	0.608	0.874	0.937	0.861	0.971	0.975				1														
20	POW	0.937	0.947	0.976	0.961	0.972	0.767	0.902	0.974	0.759	0.912	0.918					1														
21	LAW	0.915	0.937	0.935	0.869	0.755	0.939	0.947	0.744	0.898	0.904						1														
22	PP	0.930	0.939	0.918	0.814	0.620	0.940	0.653	0.844	0.871							1														
23	GB	0.953	0.851	0.725	0.903	0.977	0.797	0.931	0.937								1														
24	LOW	0.878	0.763	0.914	0.966	0.735	0.896	0.912									1														
25	CFB	0.873	0.918	0.883	0.490	0.779	0.780										1														
26	LJW	0.783	0.760	0.354	0.592	0.601											1														
27	PH	0.911	0.626	0.864	0.870												1														
28	VH	0.759	0.919	0.925													1														
29	LDPR	0.856	0.546														1														
30	LAFR	0.980															1														

Table - 21 CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS MACULATUS IN FULIKAT LAKE

PROFILES OF STROPLUS MACULATUS IN FULIKAT LAKE																																
No	Character	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CPL	EYD	HD	CHD	GH	D1A1	A1A2	CPD	POW	LAW	PP	GB	IOW	CFB	LJW	PH	VH	LDPR	LAPR	
1	TL	1	0.982	0.982	0.970	0.915	0.933	0.961	0.970	0.969	0.970	0.636	0.928	0.965	0.943	0.972	0.972	0.963	0.962	0.964	0.921	0.923	0.940	0.938	0.940	0.731	0.689	0.574	0.916	0.975	0.932	0.945
2	SL		1	0.993	0.968	0.925	0.909	0.984	0.988	0.965	0.958	0.607	0.928	0.970	0.936	0.975	0.964	0.971	0.968	0.970	0.960	0.921	0.952	0.940	0.956	0.731	0.685	0.617	0.945	0.975	0.930	0.940
3	HL			1	0.972	0.923	0.920	0.976	0.979	0.981	0.966	0.618	0.942	0.969	0.944	0.971	0.963	0.963	0.961	0.964	0.948	0.915	0.946	0.943	0.947	0.720	0.673	0.632	0.926	0.971	0.932	0.937
4	SNL				1	0.904	0.931	0.049	0.960	0.950	0.766	0.650	0.930	0.950	0.931	0.965	0.962	0.951	0.952	0.948	0.911	0.904	0.925	0.922	0.937	0.717	0.659	0.674	0.898	0.968	0.909	0.931
5	LJL					1	0.858	0.921	0.909	0.932	0.902	0.551	0.842	0.881	0.930	0.915	0.892	0.927	0.922	0.922	0.858	0.849	0.877	0.838	0.885	0.614	0.542	0.622	0.839	0.908	0.875	0.869
6	LD						1	0.875	0.908	0.893	0.952	0.669	0.923	0.931	0.928	0.932	0.941	0.890	0.894	0.893	0.824	0.874	0.880	0.869	0.882	0.709	0.889	0.741	0.828	0.925	0.908	0.903
7	LP							1	0.969	0.976	0.932	0.585	0.898	0.936	0.901	0.954	0.932	0.963	0.958	0.958	0.951	0.902	0.937	0.930	0.935	0.596	0.532	0.574	0.935	0.953	0.909	0.911
8	LVL								1	0.973	0.952	0.616	0.929	0.971	0.931	0.971	0.960	0.961	0.960	0.960	0.948	0.924	0.933	0.948	0.957	0.754	0.656	0.622	0.941	0.972	0.930	0.943
9	LA									1	0.732	0.945	0.960	0.944	0.965	0.973	0.757	0.961	0.958	0.902	0.935	0.925	0.932	0.934	0.767	0.931	0.758	0.909	0.974	0.951	0.947	
10	CPL										1	0.620	0.665	0.641	0.685	0.696	0.711	0.711	0.694	0.602	0.765	0.629	0.605	0.658	0.853	0.756	0.849	0.709	0.708	0.730	0.709	
11	CTL											1	0.944	0.945	0.935	0.938	0.903	0.905	0.905	0.884	0.876	0.929	0.911	0.875	0.703	0.655	0.699	0.860	0.935	0.913	0.900	
12	CPL												1	0.955	0.977	0.966	0.952	0.953	0.957	0.933	0.935	0.936	0.947	0.958	0.946	0.795	0.896	0.707	0.930	0.969	0.949	0.951
13	EYD													1	0.937	0.944	0.899	0.906	0.904	0.892	0.885	0.925	0.932	0.894	0.751	0.854	0.714	0.882	0.947	0.904	0.898	
14	HD														1	0.972	0.977	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	0.976	
15	CHD															1	0.959	0.957	0.964	0.914	0.942	0.930	0.945	0.946	0.772	0.907	0.720	0.923	0.970	0.950	0.945	
16	GH																1	0.994	0.994	0.947	0.948	0.945	0.945	0.952	0.787	0.904	0.705	0.955	0.971	0.947	0.953	
17	D1A1																	1	0.992	0.945	0.945	0.945	0.940	0.952	0.795	0.907	0.701	0.951	0.970	0.942	0.950	
18	A1A2																		1	0.944	0.950	0.938	0.943	0.950	0.785	0.803	0.693	0.948	0.967	0.955	0.957	
19	CPD																			1	0.879	0.982	0.941	0.925	0.749	0.810	0.567	0.945	0.931	0.900	0.890	
20	POW																				1	0.903	0.936	0.923	0.852	0.596	0.767	0.926	0.938	0.929	0.935	
21	LAW																					1	0.945	0.909	0.751	0.870	0.692	0.921	0.947	0.895	0.900	
22	PP																						1	0.941	0.842	0.845	0.719	0.963	0.951	0.923	0.976	
23	GB																							1	0.777	0.836	0.644	0.947	0.941	0.904	0.918	
24	IOW																								1	0.757	0.775	0.836	0.791	0.793	0.794	
25	CFB																									1	0.827	0.826	0.903	0.909	0.902	
26	LJW																										1	0.655	0.727	0.744	0.727	
27	PH																											1	0.936	0.905	0.898	
28	VH																												1	0.947	0.956	
29	LDPR																													1	0.946	
30	LAPR																														1	0.946

Table - 22 CORRELATION VALUES OF DIFFERENT MORPHOMETRIC CHARACTERS OF SEX POOLED SAMPLES OF ETROPLUS MACULATUS IN HYDERABAD

No	Character	WT	TL	SL	HL	SNL	LJL	LD	LP	LVL	LA	CPL	EYL	CFL	EYD	HD	CHD	GH	D1A1	A1A2	CPD	POW	LAW	PP	GB	ICW	CPB	LJW	FE	VH	LDPR	LAPE
1	WT	1	0.977	0.977	0.952	0.941	0.786	0.904	0.902	0.972	0.950	0.783	0.869	0.953	0.863	0.885	0.942	0.955	0.971	0.969	0.910	0.955	0.921	0.941	0.926	0.908	0.716	0.880	0.910	0.930	0.896	0.892
2	TL		1	0.985	0.967	0.945	0.815	0.929	0.918	0.989	0.971	0.828	0.917	0.971	0.901	0.909	0.954	0.973	0.982	0.984	0.948	0.979	0.948	0.938	0.950	0.928	0.750	0.895	0.937	0.941	0.913	0.919
3	SL			1	0.952	0.934	0.778	0.915	0.902	0.972	0.954	0.836	0.906	0.951	0.892	0.886	0.945	0.963	0.972	0.975	0.950	0.968	0.946	0.936	0.935	0.925	0.727	0.882	0.925	0.934	0.895	0.908
4	HL				1	0.961	0.782	0.905	0.918	0.968	0.949	0.804	0.882	0.941	0.858	0.892	0.928	0.935	0.945	0.948	0.905	0.944	0.903	0.938	0.926	0.853	0.725	0.873	0.909	0.926	0.891	0.893
5	SNL					1	0.754	0.890	0.925	0.951	0.915	0.792	0.862	0.916	0.845	0.876	0.903	0.903	0.912	0.924	0.876	0.932	0.885	0.927	0.929	0.885	0.743	0.854	0.892	0.885	0.884	0.883
6	LJL						1	0.740	0.791	0.822	0.826	0.664	0.779	0.761	0.766	0.743	0.803	0.795	0.787	0.896	0.747	0.821	0.780	0.763	0.776	0.769	0.623	0.815	0.762	0.792	0.722	0.751
7	LD							1	0.860	0.927	0.901	0.825	0.846	0.908	0.842	0.855	0.888	0.922	0.913	0.925	0.867	0.916	0.870	0.849	0.866	0.879	0.764	0.799	0.873	0.883	0.865	0.881
8	LP								1	0.921	0.902	0.736	0.852	0.876	0.839	0.844	0.886	0.875	0.882	0.882	0.863	0.911	0.867	0.986	0.904	0.840	0.683	0.860	0.858	0.907	0.863	0.864
9	LVL									1	0.971	0.825	0.910	0.960	0.899	0.918	0.952	0.966	0.971	0.972	0.829	0.973	0.934	0.943	0.948	0.920	0.735	0.887	0.925	0.933	0.917	0.925
10	LA										1	0.971	0.825	0.910	0.960	0.899	0.981	0.954	0.961	0.962	0.920	0.963	0.933	0.923	0.928	0.905	0.727	0.883	0.911	0.935	0.897	0.894
11	CPL											1	0.825	0.906	0.934	0.885	0.906	0.831	0.815	0.922	0.801	0.829	0.823	0.788	0.807	0.795	0.694	0.725	0.765	0.753	0.734	0.772
12	EYL												1	0.838	0.771	0.838	0.785	0.894	0.899	0.901	0.882	0.903	0.889	0.850	0.874	0.841	0.720	0.831	0.872	0.850	0.883	0.852
13	CFL													1	0.868	0.969	0.866	0.945	0.943	0.964	0.933	0.948	0.912	0.914	0.924	0.896	0.709	0.860	0.909	0.923	0.894	0.898
14	EYD														1	0.848	0.890	0.883	0.879	0.880	0.850	0.884	0.885	0.838	0.862	0.825	0.687	0.824	0.864	0.834	0.875	0.852
15	HD															1	0.889	0.900	0.903	0.901	0.849	0.909	0.872	0.882	0.870	0.854	0.646	0.820	0.857	0.883	0.880	0.859
16	CHD																1	0.932	0.943	0.941	0.910	0.945	0.931	0.898	0.905	0.903	0.701	0.886	0.874	0.916	0.884	0.883
17	GH																	1	0.986	0.992	0.917	0.952	0.925	0.916	0.903	0.909	0.716	0.858	0.915	0.944	0.887	0.896
18	D1A1																		1	0.996	0.925	0.959	0.934	0.923	0.916	0.917	0.705	0.867	0.922	0.950	0.903	0.898
19	A1A2																			1	0.937	0.963	0.934	0.920	0.917	0.922	0.721	0.870	0.924	0.950	0.901	0.903
20	CPD																				1	0.946	0.931	0.920	0.909	0.719	0.854	0.911	0.891	0.852	0.879	
21	POW																					1	0.955	0.929	0.940	0.934	0.759	0.907	0.925	0.931	0.900	0.912
22	LAW																						1	0.882	0.906	0.916	0.696	0.878	0.911	0.917	0.869	0.864
23	PP																							1	0.952	0.857	0.671	0.850	0.855	0.899	0.856	0.875
24	GB																								1	0.888	0.728	0.834	0.873	0.889	0.882	0.892
25	ICW																									1	0.707	0.835	0.895	0.899	0.843	0.829
26	CPB																										1	0.635	0.669	0.662	0.658	0.697
27	LJW																											1	0.872	0.875	0.808	0.823
28	FE																												1	0.881	0.853	0.866
29	VH																													1	0.893	0.885
30	LDPR																														1	0.931
31	LAPE																															



### Standard length (SL)

There were no significant differences in respect of the standard length between sexes except in Hyderabad samples, however it differed significantly between sexes as well as in sex pooled samples in other localities (Tables 11-13). The range of standard length recorded in sex pooled samples was 48.0-69.8 mm (mean 53.44 mm) in Cochin, 34.5-69.0 mm (mean 49.24 mm) in Muthukadu, 36.5-75.00 mm (mean 54.37 mm) in Pulikat lake and 36.0-79.5 mm (mean 50.32 mm) in Hyderabad (Table 12).

Correlations between standard length and all the characters were positive and significant in sex pooled samples of different localities (Tables 19-22).

### Head length (HL)

Differences were not significant in respect of the head length between sexes and sex pooled samples in different localities, except between sexes in Hyderabad. (Tables 11 and 12). Mean head length recorded in sex pooled sample was 18.03 mm (range 14.0-22.9 mm) in Cochin, 18.13 mm (range 12.8-23.1 mm) in Muthukadu, 18.59 mm (range 14.8-26.0 mm) in Pulikat lake and 18.70 mm (range 12.9-25.0 mm) in Hyderabad (Table 13).

Positive and significant correlations were observed between head length and all the characters of sex pooled sample in different localities (Tables 19-22).

#### Snout length (SNL)

No significant differences were observed in respect of snout length between sexes as well as in sex pooled samples of different localities (Table 11 & 12). Mean snout length recorded in sex pooled samples was 6.33 mm (range 4.2-8.2 mm) in Cochin, 6.97 mm (range 4.5-9.2 mm) in Muthukadu, 6.26 mm (range 4.8-8.5 mm) in Pulikat lake, and 7.33 mm (range 5.2-10.5 mm) in Hyderabad (Table 12).

Snout length showed positive and significant correlations with all the characters of sex pooled samples in different localities (Tables 19-22).

#### Lower jaw length (LJL)

Mean lower jaw length did not show significant difference between sexes as well as sex pooled samples of different localities (Tables 11 and 12). The range of lower jaw length varied between 3.8-7.9 mm (mean 4.35 mm) in Cochin, 2.9-4.9mm (mean 3.87 mm) in Muthukadu, 3.0-8.0 mm (mean 5.03 mm) in Pulikat lake and 3.0-5.6 mm (mean 4.23 mm) in Hyderabad (Table 12).

Correlations between lower jaw length and all the characters were positive and significant in sex pooled samples of different localities (Tables 18-21).

#### Pre-dorsal distance (LD)

There was no significant difference in respect of the pre-dorsal distance in pooled sexes in different localities. Between sexes it was significant only in Hyderabad (Tables 17 and 18). The range of pre-dorsal distance varied in pooled sexes between 17.6-30.0 mm (mean 23.10 mm) in Cochin, 16.5-29.2 mm (mean 23.28 mm) in Muthukadu, 17.2-30.10 mm (mean 23.97 mm) in Pulikat lake and 16.8-31.5 mm (mean 23.58 mm) in Hyderabad (Table 18).

Correlations between pre-dorsal distance and all the characters studied for sex pooled samples in different localities were positive and significant (Tables 18-21).

#### Pre-pectoral distance (LP)

Differences were significant between sexes in pre-pectoral distance in Hyderabad, while differences were not significant between sexes as well as in the samples of pooled out sexes in different localities. (Tables 17 and 18). Pre-pectoral distance varied in sex pooled samples between 14.9-24.8 mm (mean 19.17 mm) in Cochin, 14.8-24.8 mm (mean 19.25 mm) in Muthukadu, 14.3-26.7 mm (mean 19.72 mm) in Pulikat lake and 15.0-26.0 mm (mean 20.02 mm) in Hyderabad (Table 18).

Positive and significant correlations were observed between pre-pectoral distance and all the characters in sex pooled samples of different localities (Tables 19-22).

#### Pre-ventral distance (LVL)

Except in Hyderabad, the differences were not significant in respect of the pre-ventral distance between sexes and in sex pooled samples of different localities (Tables 17 and 18). Mean pre-ventral distance in sex pooled samples recorded was 21.86mm (range 17.0-27.3 mm) in Cochin, 21.44 mm (range 15.1-27.5 mm) in Muthukadu, 22.63 mm (range 17.5-29.2 mm) in Pulikat lake and 22.14 mm range (16.0-29.8 mm) in Hyderabad (Table 18).

Correlations between pre-ventral distance and all the characters of sex pooled samples were positive and significant in different localities (Tables 19-22).

#### Pre-anal distance (LA)

Differences were significant in respect of the pre-anal distance between sexes in Hyderabad, while it was not significant between sexes as well as in pooled out sexes in different localities. (Tables 17 and 18). The range of pre-anal distance varied between 20.8-36.5 mm (mean 27.42 mm) in Cochin, 20.2-36.8 mm (mean 27.60 mm) in Muthukadu, 20.0-40.2 mm (mean 29.16 mm) in Pulikat lake and 20.5-37.2 mm (mean 27.70 mm) in Hyderabad (Table 18).

Prenatal distance showed positive and significant correlations with all the characters studied in sex pooled samples in different localities (Tables 19-22).

Caudal peduncle length (CPL)

There was no significant difference in respect of the caudal peduncle length between sexes as well as in sex pooled samples in different localities (Tables 17 and 18). The range for caudal peduncle length of sex pooled samples varied between 3.3-5.2 mm (mean 4.62 mm) in Cochin, 2.6-6.3 mm (mean 4.39 mm) in Muthukadu, 3.2-6.2 mm (mean 4.39 mm) in Pulikat lake and 2.8-6.5 mm (mean 4.28 mm) in Hyderabad (Table 18).

Correlations were positive and significant between caudal peduncle length and all the characters of sex pooled samples in different localities (Tables 19-22).

Eye diameter (horizontal) (EYL)

Differences were not significant in respect of eye diameter between sexes and in sex pooled samples of different localities (Tables 17 and 18). Eye diameter varied in sex pooled samples between 5.0-8.0 mm (mean 5.97 mm) in Cochin, 4.0-7.8 mm (mean 5.89 mm) in Muthukadu, 4.5-8.8 mm (mean 6.16 mm) in Pulikat lake and 5.0-7.9 mm (mean 6.28 mm) in Hyderabad (Table 18).

Positive and significant correlations were observed between eye diameter (horizontal) of pooled sexes in different localities (Tables 19-22).

#### Caudal fin length (CFL)

Except in samples from Hyderabad, the differences in caudal fin length were not significant between sexes as well as in sex pooled samples in different localities (Tables 17 and 18). Caudal fin length varied in pooled out sexes between 12.9-21.0 mm (mean 17.22 mm) in Cochin, 11.2-21.5 mm (mean 16.42 mm) in Muthukadu, 14.2-22.5 mm (mean 17.17 mm) in Pulikat lake and 11.5-22.0 mm (mean 16.20 mm) in Hyderabad (Table 18).

Correlations between caudal fin length and all characters of sex pooled samples in different localities were positive and significant (Tables 19-22).

#### Eye diameter (vertical) (EYD)

There was no significant difference in respect of eye diameter (vertical) between sexes as well as in pooled out sexes of different localities (Tables 17 and 18). Mean eye diameter (vertical) was recorded in pooled out sexes as 5.81 mm (range 5.0-8.0 mm) in Cochin, 5.89 mm (range 4.5-7.7 mm) in Muthukadu, 6.05 mm (range 4.8-9.0 mm) in Pulikat lake and 6.08 mm (range 4.8-7.9 mm) in Hyderabad (Table 18).

Positive and significant correlations were observed between eye diameter (vertical) and all characters in pooled out sexes in different localities (Tables 19-22).

#### Head depth (HD)

Differences were not significant in different localities except in Hyderabad samples, in respect of head depth between sexes and pooled out sexes in different localities (Tables 17 and 18). Head depth varied in sex pooled samples between 11.30-19.00 mm (mean 14.38 mm) in Cochin, 8.9-17.8 mm (mean 14.12 mm) in Muthukadu, 11.0-20.5 mm (mean 14.83 mm) in Pulikat lake and 9.5-18.5 mm (mean 14.08 mm) in Hyderabad (Table 18).

Correlations were positive and significant between cheek depth and all the characters in pooled out sexes of different localities (Tables 19-22).

#### Cheek depth (CD)

There was no significant difference in respect of cheek depth between sexes and in pooled out sexes in samples of different localities (Tables 17 & 18). The range of cheek depth varied in the sex pooled sample between 3.2-7.8mm (mean 5.09 mm) in Cochin, 4.35-6.73 mm (mean 6.09 mm) in Muthukadu 3.1-8.8 mm (mean 5.30 mm) in Pulikat lake and 3.9-9.0 mm (mean 6.02 mm) in Hyderabad (Table 18).

Correlations were positive and significant between cheek depth and all the characters in pooled out sexes of different localities (Tables 19-22).

#### Greatest depth (GH)

Significant differences were observed in respect of greatest depth between sexes in Hyderabad, whereas, the differences were not significant between sexes in other localities and in pooled out sexes of different localities. (Tables 11 & 12). Mean greatest depth was recorded in sex pooled samples as 28.81 mm (range 21.0-37.9 mm) in Cochin, 28.46 mm (range 19.5-36.8 mm) in Muthukadu, 29.71 mm (range 22.8-37.0 mm) in Pulikat lake and 27.75 mm (range 19.2-38.2mm) in Hyderabad (Table 18).

Correlations were positive and significant between greatest depth and all the characters studied in sex pooled samples in different localities (Tables 19-22).

#### Dorso-anal depth ( $L_1A_1$ )

Except in samples of Hyderabad, differences were not significant in the dorso-anal depth between sexes and in the sex pooled samples in different localities. (Tables 17 and 18). Mean dorso-anal depth in the sex pooled samples recorded was 28.41 mm (range 20.5-38.8 mm) in Cochin, 27.31 mm (range 18.8-35.5 mm) in Muthukadu, 28.94 mm (range 26.8-37.0 mm) in Pulikat lake and 27.77 mm (range 19.2-38.2 mm) in Hyderabad (Table 18).



Dorso-anal depth showed positive and significant correlations with all the characters in the sex pooled samples of different localities (Tables 19-22).

Perpendicular anal depth ( $A_1A_2$ )

Differences were significant in respect of perpendicular anal depth between sexes in Hyderabad specimens, whereas it was not significant between sexes in other localities and in pooled out sexes in different localities (Tables 17 and 18). Mean perpendicular anal depth recorded in sex pooled sample was 28.56 mm (range 20.8-36.5 mm) in Cochin, 27.72 mm (range 19.0-35.2mm) in Muthukadu, 29.17 mm (range 23.5-36.8 mm) in Pulikat lake and 27.53 mm (range 19.2-37.6 mm) in Hyderabad (Table 18).

Positive and significant correlations were observed between perpendicular anal depth and all the characters in pooled out sexes in different localities (Tables 19-22).

Caudal peduncle depth (CPD)

There was no significant difference in respect of caudal peduncle depth between sexes as well as in pooled out sexes in different localities except in samples of Hyderabad (Tables 17 and 18). The range of caudal peduncle depth recorded varied in sex pooled samples between 6.2-11.6 mm (mean 8.39 mm) in Cochin, 6.0-12.0mm (mean 8.41 mm) in Muthukadu, 5.5-11.2 mm (mean 8.62 mm) in Pulikat lake and 6.2-12.1 mm (mean 8.44 mm) in Hyderabad (Table 18).

Caudal peduncle depth showed positive and significant correlations with all characters in sex pooled samples of different localities (Tables 19 and 21).

Snout width across pre-orbital process (PCW)

Differences were not significant in respect of snout width across pre-orbital process (PCW) between sexes as well as in sex pooled samples in different localities (Tables 11 and 12). Mean snout width across pre-orbital process in sex pooled samples recorded was 5.98 mm (range 4.7-8.0 mm) in Cochin, 5.97 mm (range 3.5-8.5 mm) in Muthukadu, 6.18 mm (range 4.5-8.5 mm) in Pulikat lake and 6.14 mm (range 4.0-9.0 mm) in Hyderabad (Table 18).

Snout width across pre-orbital process showed positive and significant correlations with all the characters in sex pooled samples of different localities (Tables 19 and 22).

Snout width across lachrymal (LAW)

There was no significant difference in respect of the snout width across lachrymal between sexes as well as in pooled out sexes in different localities (Tables 11 and 12). Range of snout width across lachrymal varied in sex pooled samples between 3.5-6.1 mm (mean 4.64 mm) in Cochin, 3.0-7.8 mm (mean 4.99 mm) in Muthukadu, 2.8-7.0 mm (mean 4.81 mm) in Pulikat lake and 3.0-7.9 mm (mean 4.93 mm) in Hyderabad (Table 18).

Positive and significant correlations were observed between snout width across lachrymal and all characters in sex pooled samples of different localities (Tables 19-21).

#### Width across pectoral fin (PP)

Except for differences between sexes in respect of width across pectoral fin in Hyderabad samples the differences between sexes in samples from other localities as well as in sex pooled samples of different localities were not significant (Tables 17 and 18). Mean width across pectoral fin recorded in sex pooled sample was 9.29 mm (range 6.8-13.0 mm) in Cochin, 8.51 mm (range 5.6-12.5 mm) in Muthukadu, 9.74 mm (range 6.5-13.0 mm) in Pulikat lake and 8.73 mm (range 6.0-12.8 mm) in Hyderabad (Table 18).

Correlations were positive and significant between width across pectoral fin and all the characters in sex pooled samples of different localities (Tables 19-22).

#### Greatest width (GB)

Differences in respect of greatest width were not significant between sexes as well as in sex pooled samples of different localities except in Hyderabad (Tables 17 and 18). The range for greatest width varied between 7.0-14.0 mm (mean 10.02 mm) in Cochin, 6.0-12.8 mm (mean 8.93 mm) in Muthukadu, 7.5-14.5 mm (mean 10.06 mm) in Pulikat lake, and 6.8-13.0 mm (mean 9.65 mm) in Hyderabad (Table 18).

Greatest width showed positive and significant correlations with all the characters in the sex pooled samples of different localities (Tables 19-28).

#### Inter-orbital width (ICW)

There was no significant difference in respect of the inter-orbital width between sexes and in pooled out sexes in different localities (Tables 17 and 18). Inter-orbital width varied in sex pooled samples between 4.5-7.9 mm (mean 5.73 mm) in Cochin, 3.5-8.7 mm (mean 6.01 mm) in Muthukadu, 4.2-8.5 mm (mean 6.29 mm) in Pulikat lake and 4.0-9.0 mm (mean 5.92 mm) in Hyderabad (Table 18).

Correlations were positive and significant between inter-orbital width and all the characters in pooled out sexes in different localities (Tables 19-28).

#### Caudal peduncle width (CPW)

Differences were not significant in respect of caudal peduncle width between sexes and in sex pooled samples in different localities (Tables 17 and 18). Mean caudal peduncle width recorded was 2.78 mm (range 1.9-4.0 mm) in Cochin, 2.65 mm (range 1.8-4.0 mm) in Muthukadu, 3.04 mm (range 1.8-5.0 mm) in Pulikat lake and 2.84 mm (range 1.8-4.0 mm) in Hyderabad (Table 18).

Caudal peduncle width showed positive and significant correlations with all the characters in sex pooled samples in different localities (Tables 19-26).

#### Lower jaw width (LJW)

Differences were not significant in respect of lower jaw width between sexes as well as in pooled out sexes in different localities (Tables 17 and 18). Lower jaw width varied in sex pooled samples between 2.9-5.7 mm (mean 4.10 mm) in Cochin, 2.2-5.8 mm (mean 3.97 mm) in Muthukadu, 2.5-6.1 mm (mean 4.29 mm) in Pulikat lake and 2.9-5.9 mm (mean 4.18 mm) in Hyderabad (Table 18).

Positive and significant correlations were found in lower jaw width and all the characters studied in sex pooled samples of different localities (Tables 19-26).

#### Length of pectoral fin (PH)

Except in respect of length of pectoral fin between sexes in the Hyderabad samples, no significant differences were observed between sexes in other localities and in pooled out sexes in different localities (Tables 17 and 18). Length of pectoral fin varied in sex pooled samples between 12.8-19.4 mm (mean 15.42 mm) in Cochin, 10.5-20.8 mm (mean 15.03 mm) in Muthukadu, 9.0-20.5 mm (mean 16.02 mm) in Pulikat lake and 11.3-20.9 mm (mean 14.80mm) in Hyderabad (Table 18).

Length of pectoral fin showed positive and significant correlations between all the characters in pooled out sexes of different localities (Tables 19-22).

#### Length of ventral fin (VH)

There were no significant differences in respect of length of ventral fin between sexes as well as in pooled out sexes samples from different localities (Tables 17 and 18). Mean length of ventral fin recorded in sex pooled sample was 10.62 mm (range 7.7-14.0 mm) in Cochin, 9.87 mm (range 7.4-11.5 mm) in Muthukadu, 11.23 mm (range 10.6-15.2 mm) in Pulikat lake and 10.28 mm (range 7.0-13.8 mm) in Hyderabad (Table 18).

Correlations were positive and significant between length of ventral fin and all the characters in sex pooled samples of different localities (Tables 19-22).

#### Longest dorsal fin ray (LDPR)

Except in respect of longest dorsal fin ray between sexes in the Hyderabad samples, the differences between sexes and in sex pooled samples were not significant in different localities (Tables 17 and 18). Mean longest dorsal fin ray recorded in sex pooled sample was 8.65 mm (range 6.0-11.8 mm) in Cochin, 8.09 mm (range 4.4-11.2 mm) in Muthukadu 9.50 mm (range 6.8-13.0 mm) in Pulikat lake and 7.81 mm (range 5.4-11.0 mm) in Hyderabad (Tables 18).

Longest dorsal fin ray showed positive and significant correlations with all the characters of sex pooled samples in different localities (Tables 13-18).

#### Longest anal fin ray (LAFR)

Differences were significant in respect of the longest anal fin ray between sexes in the Hyderabad samples, whereas they were not significant in other localities and in sex pooled samples from different localities (Table 17 and 18). The range of longest anal fin ray varied in sex pooled samples between 6.0-11.0 mm (mean 8.36 mm) in Cochin, 4.0-11.8 mm (mean 8.08 mm) in Muthukadu, 6.0-12.5 mm (mean 9.04 mm) in Pulikat lake and 4.5-11.5 mm (mean 7.81 mm) in Hyderabad samples (Table 19).

Positive and significant correlations were observed between longest anal fin ray and all the characters in sex pooled samples in different localities (Tables 19-22).

Analysis of dispersion was carried out using the method described by Rao (1974). Nine characters such as WT, SL, LVL, CPL, EYD, GH, PP, IOW and LAFR were selected. Analysis of dispersion revealed no significant differences among the localities Cochin, Muthukadu, Pulikat lake and Hyderabad for the nine characters considered.

#### 4.6. Results of studies on meristic characters of Etroneus maculatus.

##### Dorsal fin-branched rays (DFBR)

Comparison of samples between Cochin-Pulikat lake and Muthukadu-Pulikat lake did not reveal any significant difference, while significant ( $P < 0.05$ ) differences were observed between all other pairs of places (Table 23).

##### Dorsal fin-spinous rays (DFSP)

Significant differences ( $P < 0.05$ ) were observed in mean values of the samples, between all the pairs of places, except between Muthukadu and Hyderabad (Table 23).

##### Anal fin-branched rays (AFBR)

Except between Pulikat lake and Hyderabad, the differences observed in mean values of all other pairs of places were significant ( $P < 0.05$ ) (Table 23).

##### Anal fin-spinous rays (AFSP)

Significant differences were not observed between Cochin and Pulikat lake and Cochin and Hyderabad in mean values of the samples, while the differences observed in all other pairs of places were significant ( $P < 0.05$ ) (Table 23).

##### Vertebral-abdominal (VA)

A comparison of the samples between Cochin and Hyderabad, Muthukadu and Hyderabad and Pulikat lake and Hyderabad did not



TABLE - 23 SUMMARY OF THE RESULTS OF 't' TEST IN RESPECT OF MERISTIC COUNTS  
OF ETROPLUS MACULATUS BETWEEN DIFFERENT LOCALITIES

Sl. No.	LOCALITIES	Degree of Freedom	C H A R A C T E R S								
			DEPR	DESP	ADPR	ADSP	VA	VC	GRU	GRL	GT
1	Cochin Vs. Muthukadu	145	**	**	**	**	**	**	**	NS	**
2	Cochin Vs. Pulikat lake	141	NS	**	**	NS	**	**	NS	**	**
3	Cochin Vs. Hyderabad	141	**	**	**	NS	NS	**	**	NS	NS
4	Muthukadu Vs. Pulikat lake	122	NS	**	**	**	**	NS	**	**	**
5	Muthukadu Vs. Hyderabad	122	**	NS	**	**	NS	NS	NS	NS	**
6	Pulikat lake Vs. Hyderabad	118	**	**	NS	**	NS	**	**	**	NS

reveal any significant difference ( $P > 0.05$ ) in mean values, whereas, the differences observed between all other pairs of places were significant ( $P < 0.05$ , Table 23).

#### Vertebrae-caudal (VC)

Significant differences were not observed in the mean values of the samples between Muthukadu and Pulikat lake and Muthukadu and Hyderabad, while the differences observed between all other pairs of places were significant ( $P < 0.05$ ) (Table 23).

#### Gill rakers-upper (GRU)

Except between the samples from Cochin and Pulikat lake and Muthukadu and Hyderabad, the differences observed between samples of all other pairs of places were significant ( $P < 0.05$ , Table 23).

#### Gill rakers-lower 9 (GRL)

A comparison of the samples from Cochin and Muthukadu, Cochin and Hyderabad and Muthukadu and Hyderabad did not reveal any significant difference, whereas the differences observed between all other pairs of places were significant ( $P < 0.05$ , Table 23).

#### Gill teeth (GT)

Significant differences were observed in the samples of all the pairs of places, except between Cochin and Hyderabad and Pulikat lake and Hyderabad (Table 23).

A summary of the results of 't' test applied to the mean values of meristic counts is given in the table 23. It can be seen that the differences were significant in respect of more than three characters between all the pairs of localities. This suggests that the samples are derived from different stocks in all the pairs of localities.

#### 4.7. Discussion

Present morphometric and meristic studies on Etiopius suratensis lead to the conclusion that the populations of Cochin and Hyderabad and Karwar and Pondicherry are homogenous, since they did not show significant variation for both morphometric as well as meristic characters among them. Populations of Muthukadu-Mangalore, Mangalore-Pulikat lake, Muthukadu-Pulikat lake, Karwar-Goa and Goa-Pondicherry pairs of localities showed homogeneity in respect of only morphometric characters, whereas, populations of Muthukadu and Hyderabad showed homogeneity in respect of meristic characters only.

Cochin-Hyderabad and Karwar-Pondicherry although situated farthest from each other than any other localities of sample collection showed their populations as homogenous. This finding is contradictory to the statements of De Sylva et al., (1956) and Prasad (1958) that populations resemble each other more if the distribution is closer and differ more if the distance becomes greater. However, in the present study,

no consistent trend could be observed to accept or to reject the above opinions in both E. suratensis and E. maculatus.

In Cochin backwaters, water is saline, whereas, in Hyderabad it is completely fresh. Morphometric and meristic characters showed no significant variations in populations of E. suratensis, while the populations of E. maculatus varied significantly in respect of only meristic characters and morphometric characters did not show any variation. It suggests that there must be some environmental factors which are similar in both habitats which do not modify the genes responsible for meristic characters in E. suratensis, whereas, in E. maculatus certain environmental parameters might be playing a role in the variation of meristic characters.

Similarly, all the other localities although having the brackish water habitats might have some environmental factors, which play important role in modification of genes responsible for morphometric and meristic characters of both the species.

The effect of physiological and epigenetic constraints on morphology in response to certain environmental parameters, such as, temperature and oxygen are reported in *Ciscoes* (coregoninae) of the Great lakes by Todd et al., (1981).

Environmental parameters such as, temperature, salinity, pH and oxygen tension have been reported to modify the genes responsible for meristic characters (Taning, 1952; Orska, 1957; Trojnar, 1977; Durham *et al.*, 1979; Balon 1980; Todd *et al.*, 1981). Environmental changes associated with latitudes have also been demonstrated to alter the number of serially repeated characters (Gross, 1977). Thus, the phenotypic expression of characters represents a complex reciprocity between epigenetic, physiological and environmental factors.

Chervinski (1967) reported polymorphisms in respect of certain morphometric characters in two groups of a cichlid Tilapia zillii (Gervais) from Diphli river, Israel. Tilapia zillii and T. aurea from Ein-Feshkha have been reported to differ in respect of certain morphometric and meristic characters with that of T. aurea and T. zillii from Dor, Israel (Cherninski 1968 a and b) Tilapia zillii from Bardawil, a hypersaline lagoon, and from Daliyya River a fresh water body have been reported to differ in respect of certain morphometric characters (Chervinski, 1973).

Relation between number of gill-rakers or length and diet or feeding mode has frequently been reported in lake white fish Coregonus clupeaformis (Svardson, 1952, 1970, 1979; Lindsey, 1962; Bodaly 1979), and small food particle size has been reported to be generally associated with high gill raker

counts of small gill raker species in lake white fish  
Coregonus albus (Kliewer, 1970).

Thessen et al. (1981) reported that the white fish from Opeongo, which feeds substantially on zooplankton, have the highest gill raker counts, lakes Lavieille and Simco white fish have the lowest counts, while lake Ontario and South Bay white fish populations are intermediate.

Lindsay (1981) suggests that, the presence or absence of related species influences the distribution of gill raker number. He opined that gill rakers are sensitive to changes in the biological environment and, because of their key role in feeding are probably subject to rigorous selection. In the present study no such trend could be observed on specimens of both species collected from the same locality.

## 5. BIOCHEMICAL STUDIES

### 5.1. Introduction

In recent years a number of investigations have been carried out on the chemical biology of fishes to study the delicate physiological balance of biochemical constituents of fish tissues. Seasonal changes in environmental parameters such as temperature, oxygen, salinity and photoperiod, feeding and physiological state of the fish, like age and maturity directly influence the metabolic activities in the fish.

Feeding initiates somatic growth and brings primarily changes in the water, protein and fat components in the tissues with relatively less changes with respect to carbohydrates (Creach and Murat, 1974). The excess of these are stored in the body which is subsequently mobilised to the gonads during gonadal maturation. (Iles, 1984). However, proteins are not stored in the body but usually broken down to  $\text{NH}_3$  and other excretory products (Love, 1970). At times during food depletion proteins are converted to other constituents such as fat, or carbohydrate or catabolised for energy (Nagai and Ikeda, 1971).

Hence a combined study of the biochemical constituents of muscle, liver, gonad and blood at different stages of maturity would clearly present a definite picture of the mobilisation of the materials in the tissues at various times.

The pioneering work by Milery (1908) showed the changes in the chemical composition in the herring during reproductive season. This was subsequently followed by the works of Johnstone (1918), Bruce (1924), Charnon and Saby (1932) and Lovern and Wood (1937) on herring.

Blood performs most of the vital activities in the organism. This led to the belief that blood parameters can be related to the physiological state of the animals in their natural environment. Serum protein is known to take part in the maintenance of normal water content in the tissue fluids.

Usually change of temperature alters the rate of metabolism in fishes, an internal adjustment may take place to minimise the effect and keep the metabolism as near normal as possible. Farghaly *et al.*, (1973) reported in Tilapia gilli a rise in serum protein at high temperature and a decrease at low temperature, similarly, Andrews and Stickney (1972) and Thomson *et al.*, (1977) observed an increase in the total lipid with increase in temperature. However, the most striking feature exhibited by number of fishes in response to low temperature is an increase in serum glucose levels, (Nace and Schuh, 1961; Dean and Goodnight, 1964; Umringer 1967, 1969).



Leach and Taylor (1977) while studying the changes of serum glucose of Fundulus heteroclitus reported two peaks; one during the winter months which appeared to be temperature dependent and a second during the summer, correlated with the spawning season.

Murat (1976) reported wide variation in blood sugar at low temperature (below 5°C) in Cyprinus carpio and concluded that variations are due to poor turnover and absence of regulatory mechanisms.

Salinity exerts considerable influence on the biochemical composition such as the total serum proteins of the fish. Keva (1933) reported drop in the total serum protein of the migrating eels in fresh water conditions. Cordier and Bernard (1958 and 1959) reported hyperprotein with increasing salinity in stenohaline fishes Tinca tinca and Scorpaena porcus, while Farghaly et al., (1973) reported a decrease in protein content in Tilapia zilli with increasing salinity.

Environmental parameters such as photoperiod and temperatures influence annual reproductive cycle in fishes. There is an interaction between photoperiod and temperature in the regulation of some metabolites. The changes in fat and carbohydrate levels with respect to photoperiod imply that day light is involved in regulation of the rhythm (Delahunty and Devlaming, 1979).

Devlaning (1975) suggested that a long photoperiod, in combination with warm temperature regulates gonadal maturation and sharply declines the lipid reserves. On the other hand short photoperiod - warm temperature interaction have similar effect on fat levels with gonadal regression. Thus, from the above observations it is evident that changes in environment influences biochemical composition of fishes.

Majority of fishes are adapted to meet unfavourable conditions such as poor availability of food during some part of the year. During the starvation periods these fishes reduce their basal metabolic reaction and depend on the reserve material (Love, 1970). Hence they ably mobilise their body constituents during starvation. Such changes in body constituents during starvation have been studied by a number of workers (Kamra, 1966; Stirling, 1976; Narasimhamurthy et al., 1979; Jobling, 1980; Shimeno, 1982).

During starvation period an increase in moisture (Love, 1957; Idler and Bitners, 1959; Narasimhamurthy et al., 1979; Jobling, 1980) in fish tissues has been reported.

Protein is the main component which plays an important role during starvation, by mobilising from muscle and liver to provide energy for maintenance. Kamra (1966), Stirling (1976), Narasimhamurthy et al., (1979), Jobling (1980) and

Shimeno (1982) reported a decrease in proteins in various tissues during starvation.

The reduction of body and liver weight during the fasting period may partly reflect the utilisation of stored fat. Fat provides most of the energy utilized during the fasting (Shimeno, 1982). Decrease in the lipid contents has been reported in fishes during starvation (Stirling, 1976; Narasimhamurthy *et al.*, 1979; Jobling, 1980; Shimeno, 1982; and Dannevig and Norum, 1983).

The regulating mechanism of carbohydrate metabolism during starvation can be defined as the maintenance of the blood sugar value. Kamra (1966); Stirling, (1976) Narasimhamurthy *et al.*, (1979) and Shimeno (1982) reported a decrease in glucose content of blood during starvation.

Natural starvation is not always because of shortage of food, but several species seem to reduce their food intake during the final stages of maturation, perhaps because of the sheer bulk of gonads within their body cavity (Stanek, 1973; Berger and Panasenko, 1974; Mashimoto, 1976).

The constituents in the tissues are diverted initially towards growth during growing phase; which with the onset of sexual maturity in fishes results in the changes in physiology.

usually characterized by a "reproductive drain" with the diversion of material for the gonadal development. Since the levels of several constituents change at the onset of maturity, differences in the various constituents are reported between sexes. However, differences are likely to disappear at the time of the year when the gonads are inactive (Love, 1982).

The chemical effects of the sex differences have been shown in a number of instances. Low protein content has been reported in the female fishes as compared to the male fishes by Koordyl (1953), Danberg (1963), Ingram and Alexander (1977) and Alexander (1977), while the reverse condition is reported by Brooke (1964).

Jonas and Macleod (1960) observed a marked drop in total protein both in male and female sockeye salmon during spawning migration. The total proteins of the plasma has been reported to decrease with the advancement of maturity in fishes (Fassem and Siddiqui, 1970; Ochiai et al., 1975). Kristofferson et al. (1974) reported in viviparous fish Zorces viviparous that changes in plasma protein levels tend to decrease during the course of gestation and increase after parturition.

Iles (1984) opined that gonadal maturation is dependent to a large extent on the "nutritional store" rather than

the food ingested or digested, which was accumulated during the major feeding and growth period. In North sea herring, Clupea harengus it was shown that the seasonal variation exists in the major somatic components such as "lipid, water and protein" during the growth. It was shown that the proteins are accumulated in somatic tissues during the feeding and growth phases and the accumulated material is subsequently translocated to the gonads. Same observation has been made by other workers in various species (Hickling, 1930; Hoar, 1957; and Love and Robertson, 1967).

Lipid contents in the blood serum also play an important role during maturation in fishes. Melinska (1972) reported a decrease in the blood serum of maturing female Salmo trutta. Pekkarinen and Kristofferson (1975) reported in Zoarces viviparus the amount of total lipid, neutral lipid and free fatty acids being actively transported by the blood to the fetus, which increase steadily throughout pregnancy.

As such maturation involves the transfer of lipid from the liver muscle or gut to the gonad (Kuruk, 1972). A decrease of lipid in muscle as spawning approaches has been reported in various fishes (Milroy, 1903; Johnstone, 1918; Lovern and Wood, 1937; Damberg, 1963; Banerjee and Bagchi, 1969 and Pandey et al., 1976).

Chidambaram et al. (1952) and Nasurekar and Pai (1979) observed two peaks for fat contents in Labeo rohita and Cyprinus carpio, respectively and attributed one of them to the advancement of maturation and the other to voracious feeding.

Seasonal changes in the percentages of fat, water and "Crude protein" in North Sea herring occur due to active transport of water between the fish and its medium (Iles and Wood, 1965). Changes in the percentages of the three major components of somatic tissue of herring do not necessarily reflect changes in their absolute amounts. These depend also on concurrent weight changes resulting from both increases in length, defined as somatic growth and variation in "condition". This variation in condition reflects the accumulation and elimination of somatic material over and above that involved in permanent changes in length (Iles and Wood, 1965).

Hyperglycemia in connection with spawning is a well known phenomenon in vertebrates. A rise in the blood sugar in different species of fishes has been reported (Yanni, 1961; Robertson et al., 1961; Nace et al., 1964; Plack and Woodhead, 1966; Mackay and Beatty, 1968; Valtonen, 1974). Contrary to this, Jonas and Macleod (1960) and Bentley and Pollett (1965) reported a decrease in blood sugar level in Lamprolaima fluviatilis and Oncorhynchus nerka respectively.

High levels of glucose and total lipid occur in blood in flounder Platyichthys flesus before the onset of vitellogenesis (Petersen and Emerson, 1977). There was an initial decrease in protein content followed by a gradual rise till it reaches a peak at spawning and then a subsequent fall after spawning. However, Fernandes and Planas (1980) reported an increase of blood sugar at pre-spawning and post-spawning stages and a decrease during spawning.

Both glycogen and glucose have been reported to accumulate in the ovary during maturation (Greene, 1926; Yanni, 1961; Chang and Adler, 1960).

The maturation process in most species is accompanied by a general increase in the body water content as protein is removed from the muscle and liver for the building up of gonads (Love, 1970).

An increase in the water content with the advancement of maturity has been reported in many fishes (Pandey et al., 1976; Chareni, 1980; Masurekar and Pai, 1979).

Present investigation has been carried out on Etroplus suratensis and E. maculatus to establish the variations in the protein, lipid, carbohydrates, moisture and ash content occurring during gonadal maturation. Attempt has also been made to correlate these biochemical components among the

tissues. In both the species the <sup>A</sup>scale of maturity stages designated as I-V includes fishes from immature to spent stage - as described by Jayaprakash, et al., (1979) and Jayaprakash and Balakrishnan (1981).

## 5.2. Methods

### Preparation of Blood serum

The live fish was wiped free of excess water and blood was removed directly by puncturing the heart with the help of a 2 ml sterile disposable syringe. Blood was transferred to a 15 ml glass centrifuge tube and the tube was corked air tight so as to avoid any kind of haemolysis that might be caused by moisture and allowed to clot at room temperature for 40-60 minutes.

After clotting, the blood was centrifuged at 3000 rpm in an electrical centrifuge for 15 minutes and the clear serum was removed. The analysis was performed on completely transparent serum, free of any trace of haemolysis and suspended matter. Samples showing evidence of haemolysis were discarded.

The gonads were observed in every case and the maturity stage was noted. Male and female fishes were treated separately.



### Biochemical estimations

Biochemical composition in the different tissues was determined by using standard experimental methods cited in the relevant sections.

#### Moisture

A known weight of fresh tissue was allowed to dry by keeping it in an oven at 40°C till it reached a constant weight. The difference between the wet weight of the tissue and its dry weight is expressed as moisture in terms of percentage.

#### Ash

The weighed dried tissues were ashed in silica crucibles at 550°C for 6 hours in a muffle furnace and the ash percentage was determined as follows:

$$\text{Percent ash} = \frac{\text{Weight of the ash}}{\text{Weight of the dried sample}} \times 100$$

#### Protein

A known quantity of dry sample of tissue was homogenised in chloroform; methanol mixture (1:3) and the supernatant was collected. Cold 15% Trichloroacetic acid was added to the residue, homogenised and kept for 3 hours in the cold (4°C) for complete extraction of carbohydrate from the sample. The sample was then centrifuged at 1200 rpm for 10 minutes and the supernatant was collected. Re-washed the sample with cold 5% TCA, centrifuged, collected the supernatant and mixed with the

first collection of supernatant. The collected supernatant was kept for carbohydrate determination.

The residue was homogenised with 1 ml. of NaOH and kept over-night for complete dissolution. The protein content of the tissue was estimated from this extract, by the Folin-phenol method (Lowry *et al.*, 1951). The optical density of the colour developed was recorded on ECIL-UV spectrophotometer at 540 nm. A standard graph prepared with different concentration of bovine serum albumin was used, in determining the protein content of the samples. The results are expressed as percentage of wet tissue weight.

#### Carbohydrate

Carbohydrate in the tissue was determined from the supernatant of the TCA extract using modified phenol sulphuric acid method (Dubois *et al.*, 1956). The optical density (OD) was recorded on ECIL-UV spectrophotometer at 490 nm. The carbohydrate content in the tissue was determined as follows and expressed in terms of percent wet weight of tissues.

$$\text{Percent carbohydrate} = \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of sample taken}} \times 100$$

#### Total lipids

Lipid content of the tissue was determined by Bligh and Dyer (1959) method as modified by Ando *et al.*, (1977).

weighed dried samples of tissue were homogenised with chloroform:methanol (1:2) mixture for 5 minutes and kept overnight at 4°C for complete extraction of lipids. The homogenate was then centrifuged at 800 rpm for 10 minutes in cold and the supernatant was separated. The residue was mixed with chloroform and centrifuged for 5 more minutes for the complete extraction of lipids. The supernatant from the second extraction, was added to the first, together with double distilled water, so that the final solution had chloroform, methanol and water in the ratio of 2:2:1. The mixture was thoroughly shaken and allowed to settle. The upper water:methanol layer was drained off and lower lipid; Chloroform layer was dried in a desiccator with concentrated sulphuric acid as desiccant and the total lipid was estimated gravimetrically as follows:

$$\text{Percent lipid} = \frac{\text{Weight of lipid}}{\text{Weight of set tissue}} \times 100$$

#### Statistical analysis

To test the variations of biochemical components in the tissues due to gonadal maturation, the results were subjected to analysis of variance (Snedecor and Cochran, 1968). The simple and partial correlations for biochemical components between tissues were computed.

### 5.3. Results of biochemical studies on Lionelus suratensis

#### Muscle

#### Moisture

The moisture content of the muscle ranged from 73.2 to 80.3%. It was found to increase with the advancement of gonadal maturation but to decrease after spawning, when the gonad became spent (Fig. 5). In general, the females contained more moisture than the males. The overall variations in the moisture content, in different stages of maturity was statistically significant ( $P = 0.01$ ) in both males and females (Tables - 24 and 25). In females, these variations were also found to be significant at 5% level ( $P = 0.05$ ) in all the maturity stages except IV and V, while in males, a similar condition was observed in all the stages except between I and II and III and V (Table - 26 and 27).

#### Lipid

The mean lipid values of the muscle at different stages of maturity ranged from 1.62 to 2.87 in males and 0.91 to 3.06% in females (Fig. 5, Tables-26 and 27). The values decreased progressively from stage I to IV but increased in stage V for both males and females (Fig. 5). Variations in lipid content between the different stages of maturity, were found to be statistically significant at 1% level. However at 5% level the variations observed were not

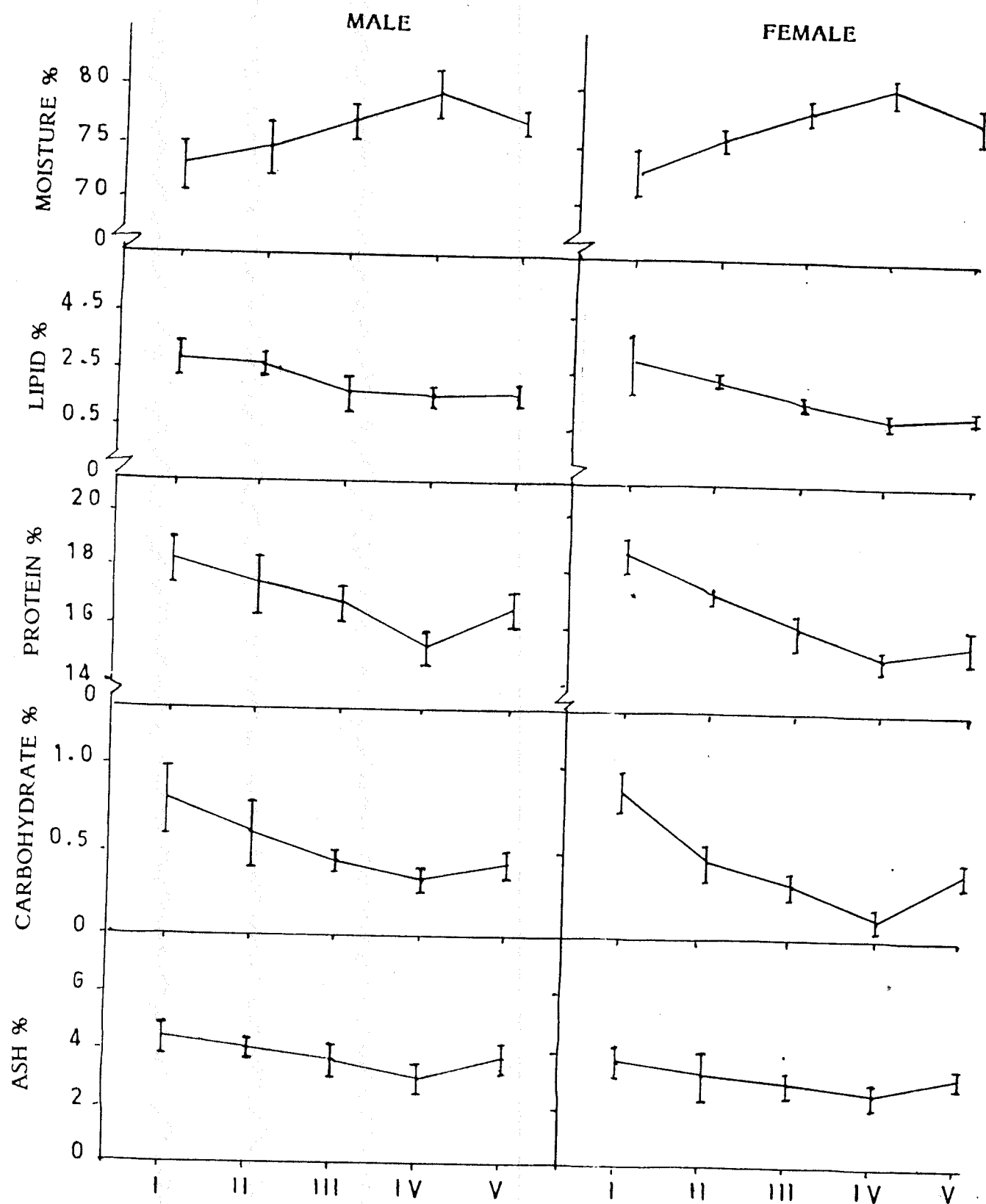


Fig.- 5. PROXIMATE COMPOSITION OF BIOCHEMICAL COMPONENTS IN MUSCLE OF ETROPLUS SURATENSIS (MALE AND FEMALE) AT DIFFERENT STAGES OF MATURITY.

TABLE - 24 ANALYSIS OF VARIANCE OF MATURITY STAGES FOR BIOCHEMICAL COMPOSITION  
OF DIFFERENT TISSUES OF ETROPLUS SURATENSIS (MALE)

Tissue	Source	Degree of freedom	Mean sum of squares of parameters				Ash
			Moisture	Lipid	Protein	Carbohydrate	
SERUM	Stage	4		0.0993 NS	3.1203 **	3175.0946 **	
	Error	41		0.0776	0.3037	26.1442	
MUSCLES	State	4	75.6355 **	4.6591 **	18.1495 **	0.4692 **	3.3232 **
	Error	60	3.1451	0.1995	0.6580	0.0265	0.2489
LIVER	Stage	4	189.7790 **	25.7643 **	16.4547 **	8.5684 **	4.5296 **
	Error	41	4.5090	0.4379	0.5183	0.1374	0.2021
TESTIS	State	4	84.1035 **	3.1379 **	6.8682 **	6.3156 **	3.3827 **
	Error	41	2.1733	0.1349	0.5129	0.8378	0.1178

NS Not significant

\*\* Significant at 1% level.

TABLE - 25 ANALYSIS OF VARIANCE OF MATURITY STAGES FOR BIOCHEMICAL COMPOSITION OF DIFFERENT TISSUES OF ETACPLUS SURATENSIS (FEMALE)

Tissue	Source	Degree of Freedom	Mean sum of squares of parameters				Ash
			Moisture	Lipid	Protein	Carbohydrate	
SERUM	Stage	4	-	0.2528 *	3.8093 **	5404.1803 **	-
	Error	46	-	0.0848	0.2374	34.1973	-
MUSCLES	Stage	4	174.8995 **	15.8508 **	44.5319 **	1.5980 **	3.4221 **
	Error	92	1.6212	0.2428	0.2632	0.0518	0.3047
LIVER	Stage	4	255.2646 **	31.7519 **	26.6685 **	13.2184 **	3.1764 **
	Error	72	2.5526	0.3737	0.2936	0.1704	0.2251
OVARY	Stage	4	480.4024 **	166.3599 **	26.9002 **	2.5034 **	7.7049 **
	Error	70	2.4952	0.9441	0.6044	0.0438	0.2894

\*\* Significant at 1% level

\* Significant at 5% level

TABLE - 26 MEANS OF BIOCHEMICAL COMPONENTS AT THE DIFFERENT STAGES OF MATURITY IN MALE ETROPLUS SURATENSIS

Tissue	Stages	Means of Biochemical Components				Ash
		Moisture	Lipid	Protein	Carbohydrate	
BLOOD SERUM	I	-	1.1617 <sup>a</sup>	4.6186 <sup>a</sup>	55.1276 <sup>a</sup>	-
	II	-	1.0969 <sup>a</sup>	3.8814 <sup>b</sup>	62.1058 <sup>b</sup>	-
	III	-	0.9309 <sup>a</sup>	3.5506 <sup>b</sup>	75.3136 <sup>c</sup>	-
	IV	-	0.9444 <sup>a</sup>	3.0645 <sup>c</sup>	98.2938 <sup>d</sup>	-
	V	-	0.9385 <sup>a</sup>	3.6192 <sup>bd</sup>	90.3898 <sup>e</sup>	-
MUSCLE	I	73.2932 <sup>a</sup>	2.8707 <sup>a</sup>	18.2897 <sup>a</sup>	0.8087 <sup>a</sup>	4.4245 <sup>a</sup>
	II	74.5881 <sup>a</sup>	2.7261 <sup>a</sup>	17.4355 <sup>b</sup>	0.6067 <sup>b</sup>	4.0445 <sup>b</sup>
	III	76.786 <sup>bd</sup>	1.7518 <sup>b</sup>	16.8284 <sup>bd</sup>	0.4242 <sup>c</sup>	3.5835 <sup>ce</sup>
	IV	79.4342 <sup>c</sup>	1.6196 <sup>b</sup>	15.1601 <sup>c</sup>	0.3268 <sup>c</sup>	3.0960 <sup>d</sup>
	V	77.0226 <sup>d</sup>	1.7581 <sup>b</sup>	16.6093 <sup>d</sup>	0.4235 <sup>c</sup>	3.6774 <sup>be</sup>
LIVER	I	59.8384 <sup>a</sup>	14.5879 <sup>a</sup>	15.8278 <sup>a</sup>	5.2935 <sup>a</sup>	4.2863 <sup>a</sup>
	II	62.9278 <sup>b</sup>	13.2736 <sup>b</sup>	15.3184 <sup>a</sup>	4.5301 <sup>b</sup>	3.5490 <sup>b</sup>
	III	66.1516 <sup>c</sup>	12.1626 <sup>c</sup>	14.2944 <sup>c</sup>	3.4851 <sup>c</sup>	3.7802 <sup>bd</sup>
	IV	71.1364 <sup>d</sup>	10.3641 <sup>d</sup>	12.6997 <sup>c</sup>	3.0297 <sup>d</sup>	2.5168 <sup>c</sup>
	V	68.3695 <sup>e</sup>	11.3990 <sup>e</sup>	13.3192 <sup>c</sup>	3.0967 <sup>d</sup>	3.5313 <sup>bd</sup>



Tissue	Stages	Means of Biochemical Components				Ash
		Moisture	Lipid	Protein	Carbohydrate	
TESTIS	I	79.3512 <sup>a</sup>	1.5430 <sup>a</sup>	15.0165 <sup>a</sup>	2.0227 <sup>a</sup>	2.0248 <sup>a</sup>
	II	77.5019 <sup>be</sup>	1.7295 <sup>ad</sup>	15.7902 <sup>b</sup>	2.4001 <sup>a</sup>	2.2497 <sup>a</sup>
	III	73.7381 <sup>c</sup>	2.2774 <sup>b</sup>	16.7004 <sup>cd</sup>	3.4056 <sup>bc</sup>	3.0820 <sup>b</sup>
	IV	72.1034 <sup>d</sup>	2.9374 <sup>c</sup>	17.2016 <sup>c</sup>	3.9860 <sup>b</sup>	3.3741 <sup>c</sup>
	V	76.4419 <sup>e</sup>	2.0312 <sup>bd</sup>	16.2115 <sup>bd</sup>	2.7147 <sup>ac</sup>	2.3377 <sup>ad</sup>

Note: Means with different superscripts differ significantly ( $P < 0.05$ )

TABLE - 27 MEANS OF BIOCHEMICAL COMPONENTS AT DIFFERENT STAGES OF MATURITY IN FEMALE ETROPLUS SURATENSIS

Tissue	Stages	Means of Biochemical Components				Ash
		Moisture	Lipid	Protein	Carbohydrate	
BLOOD SERUM	I	-	1.2342 <sup>a</sup>	4.5447 <sup>a</sup>	50.2630 <sup>a</sup>	
	II	-	1.1381 <sup>ab</sup>	3.6493 <sup>bd</sup>	65.2468 <sup>b</sup>	
	III	-	0.9445 <sup>bc</sup>	3.8254 <sup>b</sup>	78.4676 <sup>c</sup>	
	IV	-	0.8446 <sup>c</sup>	3.2618 <sup>c</sup>	108.4436 <sup>d</sup>	
	V	-	1.0530 <sup>ab</sup>	2.6970 <sup>d</sup>	91.8719 <sup>e</sup>	
MUSCLE	I	73.1617 <sup>a</sup>	3.0611 <sup>a</sup>	18.6865 <sup>a</sup>	0.8608 <sup>a</sup>	3.7022 <sup>a</sup>
	II	75.9966 <sup>b</sup>	2.3686 <sup>b</sup>	17.2269 <sup>b</sup>	0.4714 <sup>b</sup>	3.3052 <sup>b</sup>
	III	78.5292 <sup>c</sup>	1.5029 <sup>c</sup>	15.9345 <sup>c</sup>	0.3210 <sup>ca</sup>	3.0352 <sup>b</sup>
	IV	80.3208 <sup>d</sup>	0.9115 <sup>d</sup>	15.0248 <sup>d</sup>	0.1209 <sup>d</sup>	2.6218 <sup>c</sup>
	V	79.8671 <sup>d</sup>	1.2084 <sup>ce</sup>	15.4772 <sup>d</sup>	0.3986 <sup>ba</sup>	3.2352 <sup>bd</sup>

Tissue	Stage	Means of Biochemical components				Ash
		Moisture	Lipid	Protein	Carbohydrate	
LIVER	I	59.9226 <sup>a</sup>	13.0530 <sup>a</sup>	16.7829 <sup>a</sup>	5.4603 <sup>a</sup>	4.2581 <sup>a</sup>
	II	63.3072 <sup>b</sup>	12.7861 <sup>a</sup>	15.6604 <sup>b</sup>	3.8628 <sup>b</sup>	3.8176 <sup>b</sup>
	III	66.7376 <sup>c</sup>	10.9819 <sup>b</sup>	14.9812 <sup>c</sup>	3.7648 <sup>b</sup>	3.2693 <sup>c</sup>
	IV	70.8281 <sup>d</sup>	9.6604 <sup>c</sup>	13.2373 <sup>d</sup>	2.9091 <sup>c</sup>	3.0862 <sup>c</sup>
	V	67.0923 <sup>ce</sup>	11.7407 <sup>d</sup>	14.5623 <sup>ce</sup>	2.9423 <sup>c</sup>	3.3517 <sup>c</sup>
OVARY	I	78.0483 <sup>a</sup>	7.5029 <sup>a</sup>	10.2342 <sup>a</sup>	0.7281 <sup>a</sup>	2.9846 <sup>a</sup>
	II	74.4273 <sup>b</sup>	9.0445 <sup>b</sup>	11.4237 <sup>b</sup>	0.8063 <sup>a</sup>	3.7859 <sup>b</sup>
	III	67.0266 <sup>c</sup>	13.3633 <sup>c</sup>	12.8966 <sup>c</sup>	1.2855 <sup>b</sup>	4.5905 <sup>c</sup>
	IV	64.6171 <sup>d</sup>	15.1350 <sup>d</sup>	13.5988 <sup>d</sup>	1.6628 <sup>c</sup>	4.7598 <sup>c</sup>
	V	66.6790 <sup>ce</sup>	14.1525 <sup>d</sup>	12.5239 <sup>ce</sup>	1.4095 <sup>bd</sup>	3.9653 <sup>bd</sup>

Note: Means with different superscripts differ significantly. ( $P \leq 0.05$ )

significant ( $P < 0.05$ ), between stages I and II, III and IV, III and V and IV and V in males and between stages III and V in females. However at this level, the variations observed between all other stages were significant (Tables - 26 and 27).

### Protein

As in the case of moisture content, the protein content in the muscle increases with the advancement in maturation from stage I to IV and decreases in stage V in both males and females (Fig. 5). The mean protein content ranged from 15.0% to 18.6% during the process of maturation. Overall variation in protein content were found to be significant between different maturity at 1% level ( $P < 0.01$ ) (Table - 24 and 25) while at 5% level, except between the stages II and III, and III and V in males and between IV and V in females, variations observed between all other stages were statistically significant ( $P < 0.05$ , Tables - 26 and 27).

### Carbohydrate

Variations in carbohydrate content during the progress of maturation were found to be significant in males and females ( $P < 0.01$ ) (Tables - 24 and 25). The mean carbohydrate content in different stages of maturity was found to be between 0.33% to 0.80% in males and 0.12 to 0.86% in females (Tables - 26 and 27). A decrease in the carbohydrate content was observed with the advancement of maturation followed by an increase in the

spent stage for both sexes (Fig. 5). Variations were significant ( $P < 0.05$ ) between all stages except between stages III and IV, III and V, and IV and V in males and between stages II and V, and III and V in females (Tables - 26 and 27).

#### Ash

A gradual decline in the ash content was found from stage I to IV, while an increase was observed in stage V for both males and females (Fig. 5). The mean values ranged from 3.1% to 4.42% in males and 2.62 to 3.70% in females. (Tables - 26 and 27). The analysis of this parameter in different maturity stages of males and females revealed significant variations ( $P < 0.01$ ) (Tables - 24 and 25). While variations observed between stages II and V, and III and V in males and stages II and III, III and V, and II and V in females were not significant at 5% level ( $P > 0.05$ ), variations noticed between all other stages were significant ( $P < 0.05$ ), Tables - 26 and 27).

#### Liver

##### Moisture

The mean moisture content observed for liver ranged from 59.84% to 71.14% in males and 59.92% to 70.83% in females. (Tables - 26 and 27). Moisture content increased progressively from stage I to IV followed by a decrease in stage V in both sexes (Fig. 6). Overall moisture content during different maturity stages in liver revealed significant variation ( $P < 0.01$ , Tables - 24 and 25). Except between II and III,

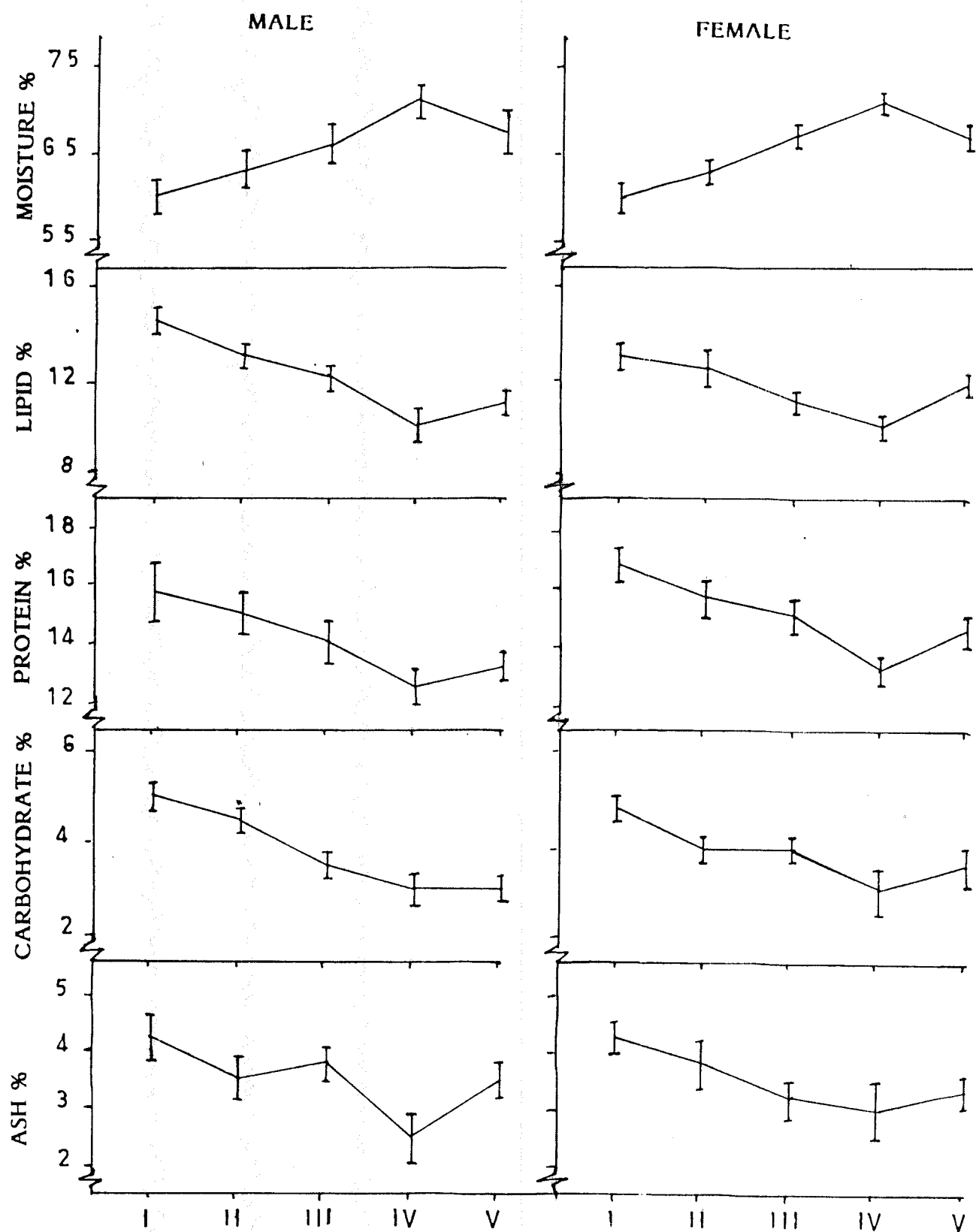


Fig.- 6. PROXIMATE COMPOSITION OF BIOCHEMICAL COMPONENTS IN LIVER OF ETROPLUS SURATENSIS (MALE AND FEMALE) AT DIFFERENT STAGES OF MATURITY.

II and V and III and V in females variations observed between all other stages of males and females were statistically significant at 5% level ( $P < 0.05$ , Tables - 26 and 27).

### Lipid

In contrast to the lipid content in the muscle, liver tissue contained more quantity of lipid. As in the case of the muscle, a decrease in the lipid content of the liver was observed from stage I to IV, followed by an increase in stage V. (Fig. 6). The mean lipid content in the liver varied from 10.36% to 14.59% in males and from 9.66% to 13.05% in females. The analysis of this parameter in overall stages of males and females revealed a significant variation ( $P < 0.01$ , Tables - 24 and 25). Variation between all stages were found to be significant ( $P < 0.05$ ) in both males and females, except between I and II stages of females (Tables - 26 and 27).

### Protein

The liver protein was also found to decrease progressively with the advancement of maturation (from stage I to IV) and was found to increase in stage V of both sexes (Fig. 6). The mean values of protein content between the stages varied from 12.70% to 15.83% in males and from 13.24% to 16.78% in females (Tables - 26 and 27). The variation observed during different maturity stages of both sexes were statistically significant at 1% level ( $P < 0.01$ , Tables - 24 and 25). At

5% level however, the variation in the protein content of the liver was found to be significant between all stages, except between I and II and IV and V in males and between stages III and V in females (Tables - 26 and 27).

### Carbohydrate

A sharp decline was observed in carbohydrate content of the liver from stage I to IV and an increase in stage V of both sexes (Fig. 6). The mean carbohydrate content observed varied from 3.03% to 5.29% in males and 2.91% to 5.46% in females (Tables - 24 and 25). Except between stages IV and V in males and stages II and III and IV and V in females the variation observed between all other stages of both sexes were significant at 5% level ( $P < 0.05$ , Tables - 26 and 27).

### Ash

Mean ash content of the liver tissue between different maturity stages varied from 2.5% to 4.29% in males and 3.10% to 4.25% in females (Tables - 26 and 27). Like the ash content of the muscle, in the liver also ash content decreased from stage I to stage IV and slightly increased in stage V in females, while in males, it seemed to fluctuate considerably with the highest value in the first stage and the lowest in the IV stage of maturity (Fig. 6). The stages II and V had the same values (3.5%), while stage III had a slightly higher value (3.7%) (Table - 26 and 27). Overall variation observed



in ash content at different maturity stages was observed to be significant, however the variations observed between the stages II and III, II and V and III and V in males and III and IV, III and V and IV and V in females were not significant at 5% level. (Tables - 26 and 27).

### Testis

#### Moisture

Considerable variation was found in moisture content of the testis. The mean moisture content of the testis ranged from 72.10% to 79.35% (Table - 26). A decrease in moisture content was observed from stage I to IV followed by an increase in the stage V of both sexes (Fig. 7). The analysis of this parameter during different maturities showed significant variation at 1% level ( $P < 0.01$ , Table - 24). While at 5% level, except between stages II and V, variations observed between all other stages of testis were significant ( $P < 0.05$ , Table - 26).

#### Lipid

An increase in the lipid content of the testis was observed from stage I to IV followed by a decrease in stage V (Fig. 7). The mean lipid content between the different stages varied from 1.54% to 2.94% (Table - 26). Overall variations observed between different maturity stages of the testis were significant ( $P < 0.01$ , Table - 24). However, the

variations noticed between the stages I and II, II and V and III and V were not significant at 5% level ( $P > 0.05$ , Tables-26).

### Protein

The mean protein content in the testis varied between 15.02% to 17.20%. An increase in protein content was observed from stage I to IV which later decreased in stage V. (Fig. 7, Table - 26). The overall variation in protein content of the testis at different maturity stage was found to be significant ( $P < 0.01$ , Table - 24). However, the variations noticed between stages II and V, III and IV and III and V were not significant (Table - 26).

### Carbohydrate

The carbohydrate content of the testis was observed to increase from stage I to IV but to decrease in stage V (Fig.7). The mean values observed ranged between 2.02% to 3.99% (Table - 26). The analysis of carbohydrate content between the different maturity stages showed a significant ( $P < 0.01$ ) variation (Table - 24). However, the variations noticed between stage I and II, I and V, II and V, III and IV and III and V were not significant at 5% level ( $P > 0.05$ ) (Table 26).

### Ash

The ash content of the testis showed an increase from stage I to IV followed by a decrease in stage V (Fig. 7). The mean values noticed varied between the stages from 2.0 to

3.4% in the testis (Table - 26). Variations observed between all the stages were significant at 1% level ( $P < 0.01$ , Table - 24) while at 5% level it was statistically significant at all stages except between stages I and II, I and V and II and V ( $P > 0.05$ , Table - 26).

### Ovary

#### Moisture

Similar to the testis, the moisture content in the ovary was also found to decrease from stage I to IV and thereafter to increase in stage V (Fig. 7). The mean values observed ranged from 64.62% to 78.0% (Table 27). The analysis of moisture content during different maturity stages showed significant variation at 1% level ( $P < 0.01$ , Table - 25). However, the variations noticed between the stages III and V were not significant at 5% level ( $P > 0.05$ , Table -27).

#### Lipid

The mean lipid content in the ovary varied from 7.5% to 15.1% between the stages (Table - 27). An increase was observed from stage I to stage IV followed by a decrease in stage V (Fig. 7). Overall variations observed during different maturity stages were found to be significant at 1% level ( $P < 0.01$ , Table - 25). However, the variation observed between the stages IV and V was not significant at 5% level ( $P > 0.05$ , Table - 27).

### Protein

In contrast to the muscle and liver, an increase in protein content in ovary was observed from stage I to stage IV, while it decreased in the stage V (Fig. 7). The mean protein content varied from 10.23% to 13.6% between the different stages of ovary (Table-27). The analysis of this parameter during different stages of maturity showed a significant variation at 1% level ( $P < 0.01$ , Table - 25). However, at 5% level the variations noticed between the stages III & V were not significant ( $P > 0.05$ , Table - 27).

### Carbohydrate

The carbohydrate content in the ovary showed an increase from stage I to IV followed by a decrease in the stage V (Fig. 7). The mean values of carbohydrate content ranged from 0.7% to 1.7% between different maturity stages (Table - 27). Overall variations in carbohydrate content observed in different maturity stages were significant at 1% level ( $P < 0.01$ , Table - 25), while at 5% level, they were not significant between the stages I-II and III-V ( $P > 0.05$ , Table - 27).

### Ash

The ash content of the ovary was found to increase from stage I to IV followed by a decrease in stage V (Fig. 7). The mean values of the ash content of the ovarian tissue ranged from 2.98% to 4.76% (Table 27). Variations observed in

different maturity stages were significant at 1% level ( $P < 0.01$ , Table - 25). However, between the stages II and V and III and IV, variations were not significant at 5% level ( $P > 0.05$ , Table 27).

### Blood serum

#### Lipid

As in the case of muscle and liver, the lipid content in the serum of female was found to decrease from stage I to IV followed by an increase in stage V, whereas in males the lipid content decreased up to stage III and it remained almost constant up to stage V. (Fig. 8). The mean values ranged between 0.93% to 1.16% in males and 0.84 to 1.23% in females (Table 26 & 27). Overall variations noticed in different maturity stages were significant at 5% level ( $P < 0.05$ ) in females, (Table 27) but were not significant in males ( $P > 0.05$ , Table - 26). However, the variations observed between the stages I and III, I and IV, II and IV and IV and V of females were significant ( $P < 0.05$ ) (Tables 26 & 27).

#### Protein

The protein content in the blood serum varied between 3.06 to 4.62 mg/100 ml in males and 2.69 to 4.54 mg/100 ml in females at different stages of maturity (Tables - 26 & 27). A decrease in protein content was observed from stage I to IV followed by an increase in stage V in males, whereas in females, protein content decreased from stage I to II

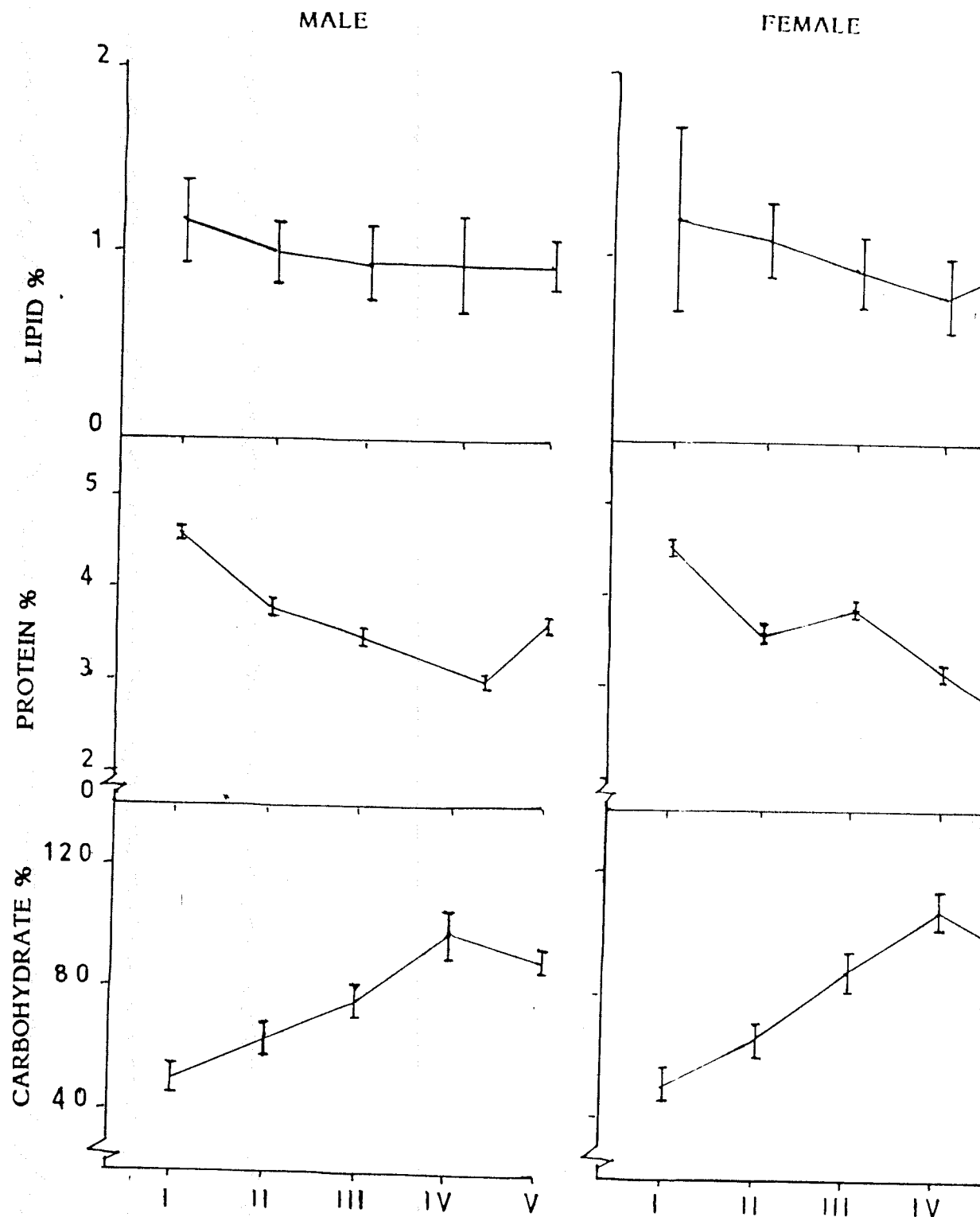


Fig.- 8. PROXIMATE COMPOSITION OF BIOCHEMICAL COMPONENTS IN BLOOD SERUM OF ETROPLUS SURATENSIS (MALE AND FEMALE) AT DIFFERENT STAGES OF MATURITY.

with a slight increase in stage III and again progressively decreased in stages IV and V (Fig. 8). Overall variations observed in different stages of maturity of males and females were significant ( $P < 0.01$ , Tables 24 and 25). However, at 5% level, the variations observed between the stage II and III, II and V and III and V in males and between stages II and III and II and V in females were not significant ( $P > 0.05$ , Tables - 26 and 27).

#### Carbohydrate

The mean carbohydrate content in different stages in blood serum ranged from 50.26 mg/100 ml to 108.44 mg/100 ml in female and 55.12 to 98.30 mg/100 ml in males (Tables 26 and 27). An increase in the carbohydrate content was observed from maturity stage I to IV followed by a decrease in stage V (Fig. 8). Variations observed in and between all the maturity stages were significant ( $P < 0.01$ ) in both sexes (Tables 24 to 27).

#### Correlation between tissues for each biochemical component of *Stroplus suratensis*

##### Male

Correlations were positive between muscle and liver for moisture, lipid, protein, carbohydrate and ash contents. There was negative correlation between muscle and testis and liver and testis in respect of moisture, lipid, protein and

carbohydrate contents. However, in respect of ash content the correlation was negative between muscle and testis only, liver and testis were positively correlated. Positive correlations were observed between muscle and liver with that of blood serum, in respect of lipid and protein contents whereas for carbohydrates it was negative between them. There were negative correlations in respect of lipid and protein, while correlations were positive in respect of carbohydrate contents between testis and serum. All these correlations were statistically significant (Table 28).

Partial correlation values show that the correlation between testis and muscle for moisture content is negative and statistically significant ( $P < 0.01$ ), while between testis and liver it is positive and significant. For ash content the partial correlation was found to be negative and significant between testis and muscle ( $P < 0.01$ ), but positive and not significant between testis and liver ( $P > 0.05$ , Table 29).

Thus, it can be inferred from these partial correlations, that there is a close association between the moisture content of the testis with that of the muscle and liver. Hence, fluctuations in the moisture content of the testis bring about appreciable changes in the moisture content of the muscle and liver. The ash content of the testis, however, seems to be more closely related to that of the muscle as compared to the liver.



TABLE - 28 CORRELATION BETWEEN TISSUE FOR EACH BIOCHEMICAL COMPONENT IN  
ETROPLUS OCHOTENSIS (MALE) (n=15)

Parameter	Tissue	Muscle	Liver	Testis	Blood serum
Moisture	Muscle	1	+ 0.9855 **	- 0.9312 **	
	Liver		1	- 0.8676 **	
	Testis			1	
Lipid	Muscle	1	+ 0.9254 **	- 0.8428 **	+ 0.9031 **
	Liver		1	- 0.9024 **	+ 0.8586 **
	Testis			1	- 0.7734 **
	Serum				1
Protein	Muscle	1	+ 0.9455	- 0.9411 **	+ 0.9588 **
	Liver		1	- 0.8714 **	+ 0.8981 **
	Testis			1	- 0.9770 **
	Serum				1
Carbohydrate	Muscle	1	+ 0.9798	- 0.8870 **	- 0.9196 **
	Liver		1	- 0.8150 **	- 0.9485 **
	Testis			1	+ 0.8064 **
	Serum				1
Ash	Muscle	1	+ 0.8811	- 0.9104 **	
	Liver		1	+ 0.7376 **	
	Testis			1	

TABLE - 29 PARTIAL CORRELATION COEFFICIENTS BETWEEN TISSUES FOR EACH BIOCHEMICAL COMPONENT IN ETROPLUS SURATENSIS (MALE)

Correlation	Moisture	Lipid	Protein	Carbohydrate	Ash
ry 1.2	- 0.9012 *	-	-	-	- 0.8157 *
ry 2.1	+ 0.8068 *	-	-	-	+ 0.3299 NS
ry 1.23	-	- 0.3037 NS	- 0.1590 NS	- 0.8145 *	-
ry 2.13	-	- 0.6409 *	+ 0.1559 NS	+ 0.6972 *	-
ry 3.12	-	+ 0.5870 *	- 0.7708 *	+ 0.4688 NS	

NS : Not significant; \*\* : Significant at 1% level; \* : Significant at 5% level

ry 1.2 : correlation between testes & muscle keeping liver constant

ry 2.1 : correlation between testes & liver keeping muscle constant

ry 1.23: correlation between testes & muscle keeping liver & serum constant

ry 2.13: correlation between testes & liver keeping muscle & serum constant

ry 3.12: correlation between testes & serum keeping muscle & liver constant

1. Muscle

2. Liver

3. Serum

4. Testis or Ovary

The partial correlation observed for lipid was significantly negative between testis and liver, but significantly positive between testis and serum. Between testis and muscle, however, the partial correlation was negative but not significant. It may be concluded that the effect of the lipid content of the liver and serum camouflaged over the effect of the lipid of muscle. In the mobilization of lipid to the testis, liver and serum seem to be more involved than muscle.

Partial correlation coefficient of protein was found to be significantly negative between testis and serum ( $P < 0.05$ , Table 29). Although there was a positive correlation between testis and liver and a negative correlation between testis and muscle, these were not significant statistically. Hence, the effect of serum protein is camouflaged over the effect of muscle and liver protein. Thus, serum protein seems to be more involved in mobilization to the testis as compared to muscle and liver proteins.

An estimate of the partial correlation coefficient of carbohydrate indicated that between testis and muscle there was a significantly negative correlation, while between testis and liver there was a significantly positive correlation. Between testis and serum, though the correlation is positive, it was not significant. The effect of the muscle and liver carbohydrate have camouflaged the effect of the serum carbohydrate, indicating that carbohydrates of the muscle and liver

are more closely related to gonadal maturation than carbohydrate of the serum.

### Females

As in the case of males, muscle and liver in the females showed the same trend of correlation for all biochemical components studied. The correlations for moisture, lipid, protein, carbohydrate and ash between muscle and liver were found to be positive and significant ( $P < 0.01$ ), whereas between muscle and ovary and between liver and ovary, the correlations were negative and significant ( $P < 0.01$ ) for all the parameters (Table 30). The lipid and protein contents of the muscle and liver with that of serum had positive correlations, while the carbohydrate had negative correlation. Between ovary and serum the correlation was positive for protein and carbohydrate and negative for lipid (Table 30). All these correlations were statistically significant ( $p < 0.01$ ).

Partial correlation coefficients of moisture content was significantly positive between ovary and muscle, whereas positive but not significant between ovary and liver. An estimate of the same for ash content indicated that there was a significantly negative correlation, between ovary and muscle and ovary and liver (Table 31). This showed that, the moisture content of the ovary was closely related to that of muscle, while ash content of the ovary was closely related to both muscle and liver.

TABLE - 30 COMPARISON BETWEEN TISSUES FOR EACH BIOCHEMICAL COMPONENT IN  
ETROPLUS SURATENSIS (FEMALE) (n=15)

Parameter	Tissue	Muscle	Liver	Ovary	Blood serum
Moisture	Muscle	1	+ 0.9628 **	- 0.9831 **	-
	Liver		1	- 0.9679 **	-
	Ovary			1	-
Lipid	Muscle	1	+ 0.8817 **	- 0.9915 **	+ 0.8059 **
	Liver		1	- 0.8932 **	+ 0.9825 **
	Ovary			1	- 0.9057 **
	Serum				1
Protein	Muscle	1	+ 0.9525 **	- 0.9740 **	+ 0.8337 **
	Liver		1	- 0.9533 **	+ 0.7518 **
	Ovary			1	+ 0.6918 **
	Serum				1
Carbohydrate	Muscle	1	+ 0.9095 **	- 0.8687 **	- 0.9102 **
	Liver		1	- 0.8471 **	- 0.9158 **
	Ovary			1	+ 0.9728
	Serum				1
Ash	Muscle	1	+ 0.9202 **	- 0.9559 **	-
	Liver		1	- 0.9563 **	-
	Ovary			1	-

TABLE - 31 PARTIAL CORRELATION BETWEEN TISSUES FOR EACH BIOCHEMICAL COMPONENTS IN ETROPLU SUBATENSIS (FEMALE)

Correlations	Moisture	Lipid	Protein	Carbohydrate	Ash
ry 1.2	+ 0.7540 *				- 0.6633 *
ry 2.1	+ 0.4320 NS				- 0.6670 *
ry 1.23		- 0.9635 *	- 0.9825 *	- 0.0514 NS	
ry 2.13		- 0.4738 NS	- 0.4591 NS	0.4464 NS	
ry 3.12		+ 0.3974 NS	+ 0.9655 *	0.9034 *	

\* Significant at 5% level

NS Not significant

Lipid content in ovary and muscle and ovary and liver showed negative partial correlation, but it was positive between ovary and serum. These coefficients were significant ( $P < 0.01$ ) between ovary and muscle only. Here the effect of lipid content of muscle camouflaged over the effect of lipid content of liver and serum, which indicated the close relation of lipid with muscle as compared to that of liver and serum, where correlations were not significant (Table 31).

Partial correlation coefficients for protein were found to be negative between ovary and muscle and ovary and liver, while it was positive between ovary and serum, but were significant only ( $P < 0.01$ ) between ovary and muscle and ovary and serum. It shows that the effect of protein content of muscle and serum camouflaged over the effect of liver protein content, hence it can be inferred that the mobilisation of protein content is more closely related to muscle and serum than the liver.

The partial correlation coefficients were positive for carbohydrate between ovary and liver and ovary and serum and negative between ovary and muscle. The coefficients were significant between ovary and serum only ( $P < 0.01$ , Table 31). Here also the effect of serum carbohydrate camouflaged over the effect of muscle and liver carbohydrate. It indicates that the mobilisation of carbohydrate content is more from serum to ovary than from muscle and liver.

#### 5.4. Results of biochemical studies on Strepplus regulatus

The estimation of the biochemical parameters was carried out only in the females of this species due to non-availability of adequate quantity of testis tissue in different maturity stages.

##### Muscle

##### Moisture:

The moisture content in the muscle increased from stage I to IV, but decreased in stage V (Fig. 9). The mean values obtained ranged between 74.3% - 79.9% in females of different maturity stages (Table 33). The analysis of variance of moisture content in different maturity stages was found to be significant ( $P < 0.01$ ) (Table 32). However, at 5% level the variations obtained between the stages II and V and III and IV were not significant ( $P > 0.05$ ) (Table 33).

##### Lipid

The mean lipid content in the muscle varied from 0.8% to 1.9% in the different stages of maturity (Table 33). A decrease in the lipid content was observed with the advancement of maturity upto stage IV and thereafter an increase in stage V. (Fig. 9). Overall variations observed in different maturity stages were significant ( $P < 0.01$ , Table 32), however, the variations obtained between the stages III and IV, III and V, and IV and V were not significant ( $P > 0.05$ , Table 33).



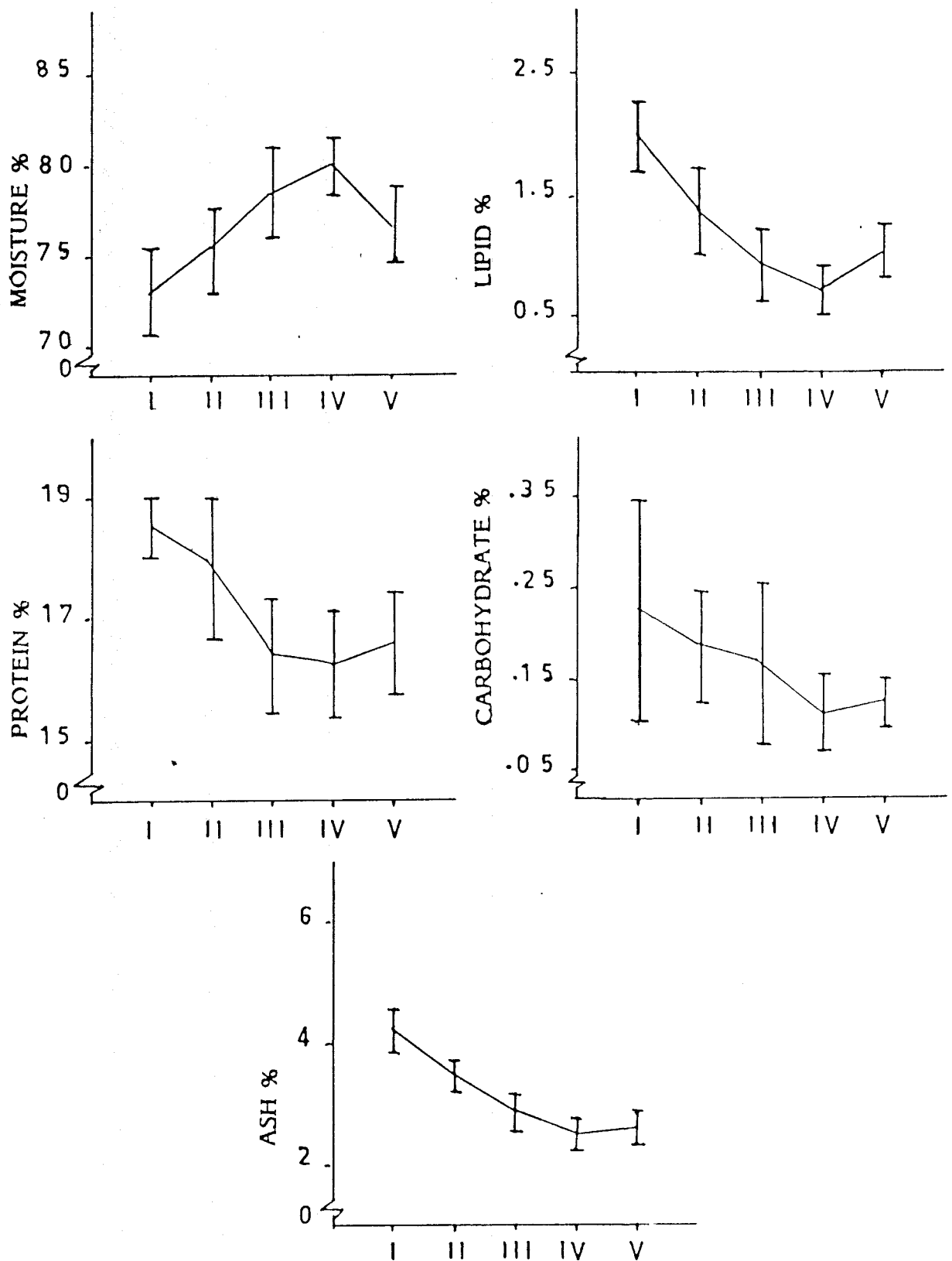


Fig. 9. PROXIMATE COMPOSITION OF BIOCHEMICAL COMPONENTS IN MUSCLE OF ETROPLUS MACULATUS (FEMALE) AT DIFFERENT STAGES OF MATURITY:

TABLE - 32 - ANALYSIS OF MATURITY STAGES FOR BIOCHEMICAL COMPOSITION OF  
DIFFERENT TISSUES OF ETROPLUS MACULATUS (FEMALE)

Tissue	Source	Degree of freedom	Mean sum of squares of parameters				Ash
			Moisture	Lipid	Protein	Carbohydrate	
MUSCLE	Stage	4	75.2397**	2.9996 **	13.2227 **	0.0316 **	9.8829 **
	Error	79	2.8509	0.0777	0.9485	0.0058	0.2224
LIVER	Stage	4	190.9137**	32.9507 **	24.5786 **	0.0674 **	5.8052 **
	Error	63	1.7968	0.2882	0.4317	0.0046	0.0629
OVARY	Stage	4	423.1348**	74.1778 **	104.0026 **	0.1299 **	7.6815 **
	Error	62	10.1876	0.6473	0.2856	0.0007	0.0789

\*\* Significant at 0.1% level

TABLE - 33 MEANS OF BIOCHEMICAL COMPONENTS AT DIFFERENT STAGES OF MATURITY  
IN FEMALE ETROPLUS MACULATUS.

Tissue	Stages	Means of biochemical components				Ash
		Moisture	Lipid	Protein	Carbohydrate	
MUSCLE	I	74.2628 <sup>a</sup>	1.9621 <sup>a</sup>	18.4909 <sup>a</sup>	0.2062 <sup>a</sup>	4.7822 <sup>a</sup>
	II	76.2296 <sup>b</sup>	1.3543 <sup>b</sup>	17.7547 <sup>a</sup>	0.1559 <sup>a</sup>	4.2029 <sup>b</sup>
	III	78.8961 <sup>c</sup>	0.8912 <sup>c</sup>	16.4274 <sup>b</sup>	0.1399 <sup>ab</sup>	3.2753 <sup>c</sup>
	IV	79.8636 <sup>c</sup>	0.8266 <sup>c</sup>	16.2571 <sup>b</sup>	0.0860 <sup>c</sup>	2.7591 <sup>d</sup>
	V	77.1814 <sup>bd</sup>	0.9706 <sup>c</sup>	15.6594 <sup>b</sup>	0.1041 <sup>bc</sup>	3.1954 <sup>c</sup>
LIVER	I	66.4415 <sup>a</sup>	13.6177 <sup>a</sup>	15.1636 <sup>a</sup>	0.2958 <sup>a</sup>	4.6124 <sup>a</sup>
	II	69.1245 <sup>b</sup>	12.0690 <sup>b</sup>	13.9446 <sup>b</sup>	0.2105 <sup>b</sup>	3.9532 <sup>b</sup>
	III	72.1919 <sup>c</sup>	11.0847 <sup>c</sup>	13.0890 <sup>c</sup>	0.1846 <sup>d</sup>	3.3696 <sup>c</sup>
	IV	75.4455 <sup>d</sup>	9.6704 <sup>d</sup>	11.6409 <sup>d</sup>	0.1100 <sup>c</sup>	2.9762 <sup>d</sup>
	V	75.0461 <sup>d</sup>	10.0260 <sup>d</sup>	12.4544 <sup>e</sup>	0.1352 <sup>bc</sup>	3.0923 <sup>d</sup>

Tissue	Stage	Means of biochemical components				Ash
		Moisture	Lipid	Protein	Carbohydrate	
OVARY	I	79.5523 <sup>a</sup>	7.4940 <sup>a</sup>	9.2902 <sup>a</sup>	0.0474 <sup>a</sup>	2.9192 <sup>a</sup>
	II	74.4592 <sup>b</sup>	9.9047 <sup>b</sup>	11.3758 <sup>b</sup>	0.0729 <sup>b</sup>	3.6731 <sup>b</sup>
	III	69.6081 <sup>c</sup>	11.7782 <sup>c</sup>	13.4767 <sup>c</sup>	0.0842 <sup>b</sup>	4.2835 <sup>c</sup>
	IV	64.3744 <sup>d</sup>	13.7929 <sup>d</sup>	16.2546 <sup>d</sup>	0.1341 <sup>c</sup>	5.0048 <sup>d</sup>
	V	68.1822 <sup>bc</sup>	12.2086 <sup>c</sup>	15.4636 <sup>b</sup>	0.1175 <sup>c</sup>	4.1217 <sup>c</sup>

Note: Means with different superscripts differ significantly ( $P < 0.05$ )

### Protein

The protein content decreased with the increasing maturity upto stage IV, but showed an increase in stage V (Fig. 9). The mean values for protein content in the muscle varied from 16.25% to 18.5% (Table 33). The analysis of protein content in different maturity stages showed significant ( $P < 0.01$ ) variation (Table 32). However at 5% level the variations observed between the stages I and II, III and IV, III and V and IV and V were not statistically significant ( $P > 0.05$ ) (Table 33).

### Carbohydrate

As in the case of protein, the carbohydrate content in the muscle decreased from stage I to III and increased in stage V (Fig. 9). The mean values varied from 0.087% to 0.21% between different maturity stages (Table 6). The carbohydrate content of the muscle varied significantly ( $P < 0.01$ ) in different maturity stages (Table 32). However, at 5% level the variations between the stages I and IV, I and V, II and IV, II and V and III and IV were significant ( $P < 0.05$ ), those observed between all other stages were not significant ( $P > 0.05$ , Table 33).

### Ash

The ash content of the muscle tissue showed the same trend of fluctuation as in the case of lipid, protein and carbohydrate (Fig. 9). The mean ash content ranged from 2.8%

to 4.6% between the stages. The ash content varied significantly ( $P < 0.01$ ) in different stages (Table 32). At 5% level variations observed between all stages except between III & V were significant ( $P < 0.05$ , Table 33).

### Liver

#### Moisture

The moisture content in the liver was found to increase from stage I to IV, followed by a decrease in the stage V (Fig. 10). The mean moisture content ranged from 68.44 to 75.44% between the different stages. Variations in the different maturity stages were found to be significant ( $P < 0.01$ , Table 33). However, at 5% level between the stages IV and V variations obtained were not significant ( $P > 0.05$ , Table 33).

#### Lipid

The mean lipid content in the liver ranged from 9.7% to 13.6 between different stages (Table 33). The lipid content showed a decrease from stage I to IV followed by an increase in stage V (Fig. 10). The analysis of this parameter revealed significant ( $P < 0.01$ , Table 32) variation between the stages. However, variations were not significant ( $P > 0.05$ ) between stage IV and V and 5% level (Table 33).

#### Protein

A decrease was observed in the protein content of the liver from stage I to IV but in stage V it showed an increase

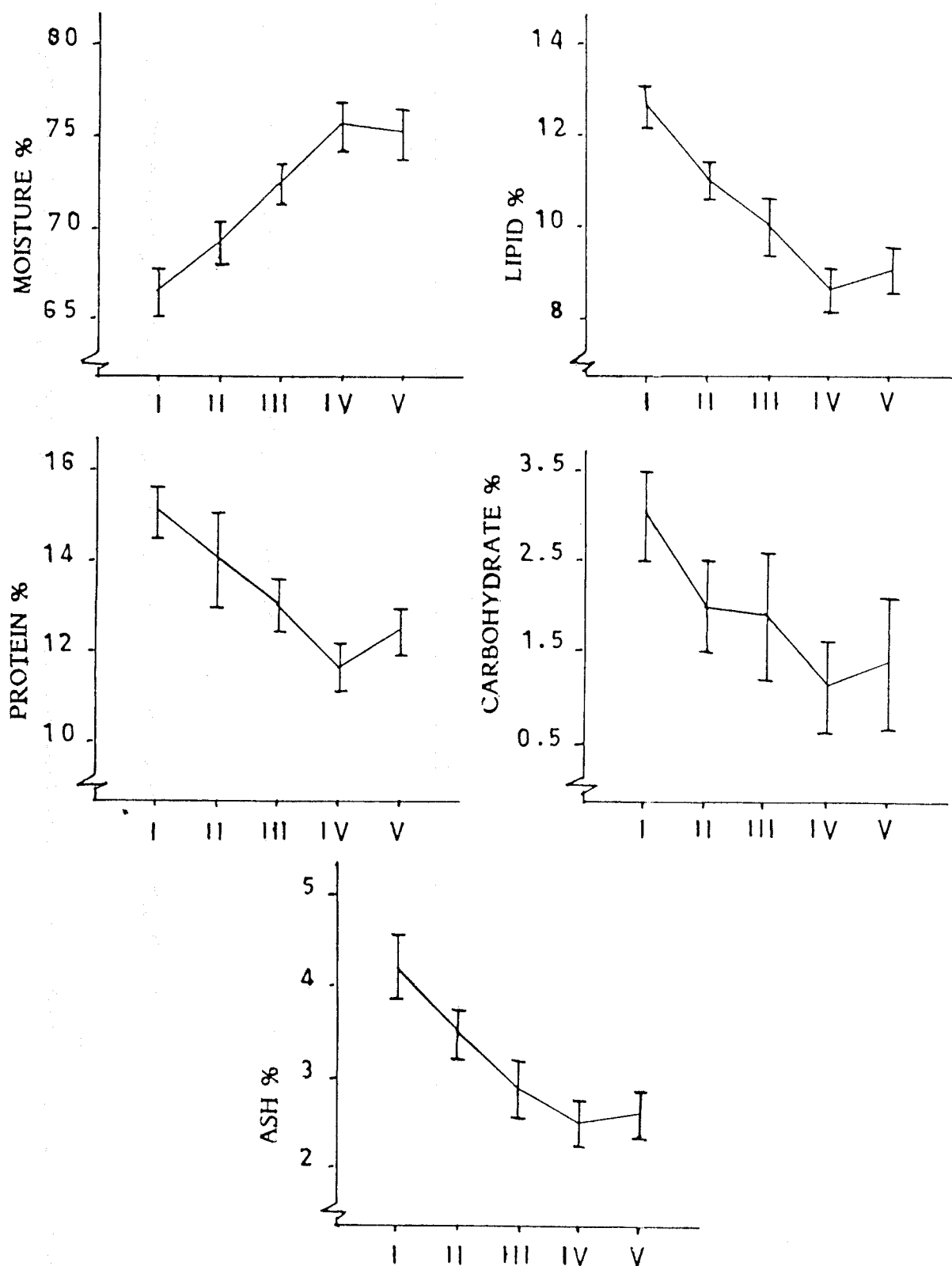


Fig.- 10. PROXIMATE COMPOSITION OF BIOCHEMICAL COMPONENTS IN LIVER OF ETROPLUS MACULATUS(FEMALE) AT DIFFERENT STAGES OF MATURITY.

(Fig. 10). The mean values ranged from 11.64 to 15.2% between the various stages. Variations in overall protein content in different stages (Table 32) and between the stages were significant ( $P < 0.01$ , Tables 32 and 33).

#### Carbohydrate

The carbohydrate content in liver showed a decreasing trend from stage I to IV followed by an increase in stage V (Fig. 10). The mean values varied from 0.11 to 0.3% between the stages (Table 33). The analysis of this parameter showed significant ( $P < 0.01$ ) variation in the different stages (Table 32). At 5% level variations observed between all stages, except between stages II and III, II and V, III and V and IV and V were significant ( $P < 0.05$ , Table 33).

#### Ash

The ash content of the liver showed a similar trend of decrease from stage I to IV and increase in Stage V (Fig. 10). The mean ash content ranged from 2.97 to 4.6% (Table 10). The variation was significant in different stages ( $P < 0.01$ , Table 32). However, the variations between stages IV and V were not significant at 5% level ( $P > 0.05$ , Table 33).

#### Ovary

##### Moisture

Contrary to the situation in muscle and liver, the moisture content in the ovary decreased with the advancement



of maturation (Stage I to IV) and increased in stage V (Fig.11). The mean moisture content ranged from 64.37% to 79.55% between the stages. The overall moisture content in the different maturity stages was found to vary significantly ( $P < 0.01$ , Table 32). However, between stages II and V and III and V the variations were not significant ( $P > 0.05$ , Table 33).

#### Lipid

The mean values for lipid content in the ovary ranged from 7.49% to 13.79% between the stages (Table 33). An increase in lipid content was observed from stage I to IV, followed by a decrease in stage V (Fig. 11). The lipid content in the ovary varied significantly in different stages ( $P < 0.01$ , Table 32). Except between stage III & V, the variations observed between all other stages were significant ( $P < 0.05$ , Table 33).

#### Protein

An increase in protein content of the ovary was observed from stage I to IV followed by a decrease in stage V (Fig. 11). The mean protein content ranged from 9.3% to 16.25% between the stages. The analysis of protein content in different maturity stages showed significant variation ( $P < 0.01$ , Table 32). The variations were also found to be significant between all the stages at 5% level ( $P < 0.05$ , Table 33).

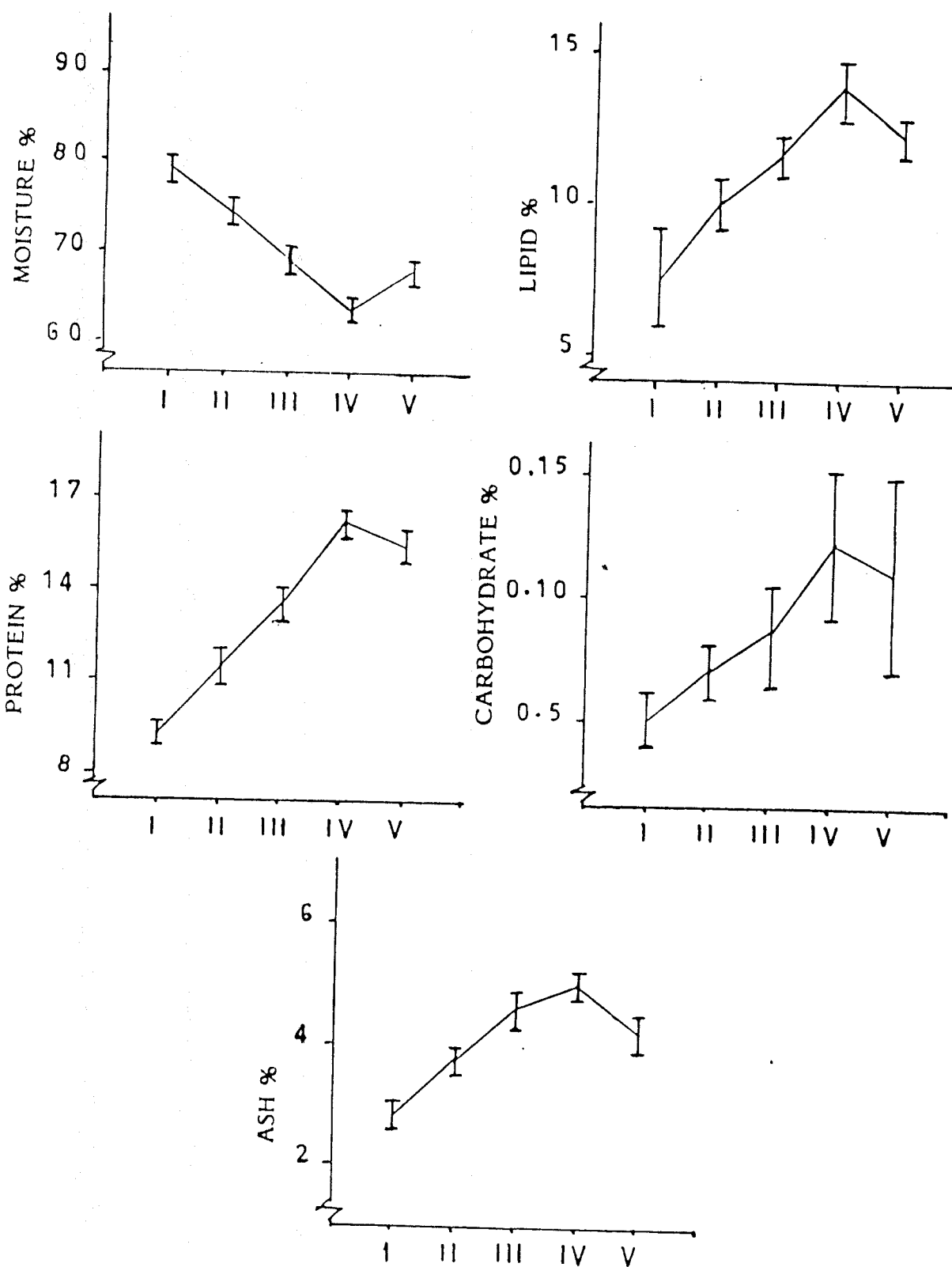


Fig.11. PROXIMATE COMPOSITION OF BIOCHEMICAL COMPONENTS IN OVARY OF ETROPLUS MACULATUS AT DIFFERENT STAGES OF MATURITY.

### Carbohydrate

The carbohydrate content in the ovary increased from Stage I to IV, while it decreased in stage V (Fig. 11). The mean carbohydrate content varied from 0.04% to 0.1% between the stages (Table 33). The overall carbohydrate content revealed significant variation ( $P < 0.01$ ) in different stages (Table 32). However, at 5% level the variations between the stages II and III and II and V were not significant ( $P > 0.05$ , Table 33).

### Ash

As in the case of other parameters, the ash content in the ovary increased progressively from stage I to IV and in stage V it decreased (Fig. 11). The mean ash content varied from 2.9% to 5.0% between the stages (Table 33). This parameter showed significant variation in different stages ( $P < 0.01$ , Table 32). At 5% level the variations between all stages except between the stages III and V were significant ( $P < 0.05$ , Table 33).

### Correlation between tissues for each biochemical component of *Etroplus maculatus* (Female)

Correlation between muscle and liver was observed to be positive, while between muscle and ovary, and liver and ovary it was negative for all biochemical components, namely, moisture, lipid, protein, carbohydrate and ash content (Table 34).

TABLE - 34 CORRELATION BETWEEN TISSUES FOR EACH BIOCHEMICAL COMPONENTS IN  
PERIPLUS MACULATUS (n=15)

Biochemical components	Tissues	Muscle	Liver	Ovary
Moisture	Muscle	1	+ 0.8412 **	- 0.9335 **
	Liver		1	- 0.9753 **
	Ovary			1
Lipid	Muscle	1	+ 0.9415 **	- 0.9654 **
	Liver		1	- 0.9820 **
	Ovary			1
Protein	Muscle	1	+ 0.9401 **	- 0.9347 **
	Liver		1	- 0.9908 **
	Ovary			1
Carbohydrate	Muscle	1	+ 0.9985 **	- 0.9903 **
	Liver		1	- 0.9826 **
	Ovary			1
Ash	Muscle	1	+ 0.9858 **	- 0.9659 **
	Liver		1	- 0.9359 **
	Ovary			1

TABLE - 35 PARTIAL CORRELATION COEFFICIENTS BETWEEN TISSUES FOR EACH BIOCHEMICAL COMPONENTS IN FEMALE ETROPIDAE MACULATUS

Correlation	Moisture	Lipid	Protein	Carbohydrate	Ash
ry 1.2	- 0.9468 *	- 0.6417 *	- 0.0704 NS	- 0.9021 *	- 0.7318 *
ry 2.1	- 0.9801 *	- 0.8315 *	- 0.9251 *	+ 0.8169 *	+ 0.3745 NS

\*\* : Significant at 1% level

\* : Significant at 5% level

NS : Not significant

ry 1.2: Correlation between ovary and muscle keeping liver constant.

ry 2.1: Correlation between ovary and liver keeping muscle constant.

Partial correlation coefficients were found to be negative for moisture, lipid, protein, carbohydrate and ash content, between ovary and muscle. Similarly, the partial correlation coefficients for moisture, lipid and protein contents were negative between liver and ovary, but for carbohydrate and ash contents positive. These correlations were significant ( $P < 0.01$ ) between ovary and that of muscle and liver for moisture, lipid and carbohydrate (Table 35). It was also significant ( $P < 0.01$ ) for protein content between ovary and liver and for ash content between ovary and muscle.

From the above, it can be inferred that the mobilisation of moisture, lipid and carbohydrate is closely linked in ovary muscle and liver. Similarly, mobilisation of protein content is more closely related in ovary and liver than between ovary and muscle. However, the fluctuation of the ash content of the ovary is more closely related to that of muscle than to the liver.

### 5.5. Discussion

There is very little information on biochemical studies related to reproductive physiology in the Indian cichlids, E. suratensis and E. maculatus. Present investigations have been carried out to establish the variations found in the moisture, lipid, protein, carbohydrate and ash content of different tissues during maturation in E. suratensis and E. maculatus.

### Moisture

Water as a body component of the fish plays a major part in the changes taking place during processing and storage. Water participates in the biochemical reactions and diffusion process of the fish body. The water content of the fish body varies within limited range in different species of fishes. In Hilsa ilisha, it is very low (53.7%) while in Cyprinus carpio (88.3%) and Tinca tinca (90.2%), it is comparatively high (Anon, 1962). In the present study, the moisture content in muscle tissue of E. suratensis is found to range from 73.16% to 80.32% with slight difference in male and female. Moisture content in female E. maculatus ranged between 74.26% to 79.8%. Differences in moisture content between male and female has been observed in cat fish Heteropneustes fossilis (Shreni, 1980). Damberg (1963) reported that water content increases significantly during the time of spawning in Gadus morhua. An increase in the moisture content of the muscle has been reported in Cyprinus carpio (Masurekar and Pai, 1979) and H. fossilis (Shreni, 1980) with the advancement of maturity, followed by a decrease after spawning. A similar trend in moisture content in muscle of E. suratensis and E. maculatus has been observed in the present study.

The moisture content in the liver was reported to range between 62.0 to 80.9% in Oncorhynchus kisutch (MacLeod et al., 1960), O. nerka (Idler and Bitners, 1960), H. Fossilis

(Shivani, 1980), and E. suratensis (Varghese, 1983).

From the present study, it is found that the moisture content in liver of E. suratensis and E. maculatus ranged from 59.83% to 71.13% and 66.4% to 75.4% respectively. Differences in moisture content of liver between the sexes have been observed in E. suratensis. Shivakami (1980) has reported differences in moisture level of liver between sexes in Asperra daniconius. A significant increase in water content was observed in the liver as spawning approached and after spawning it was observed to decrease in both male and female E. suratensis and female E. maculatus. This is in full agreement with the observations on E. suratensis by Varghese (1983) who suggested that the moisture content in liver of E. suratensis increases significantly during gonadal development and decreases after spawning.

Moisture content in the ovary and testis of C. nerka has been reported to range from 56.1 to 62.4% and from 80.1 to 80.5% respectively (Idler and Bitners, 1960). In the present study, moisture content in the ovary has been found to range from 64.61 to 78.04% in E. suratensis and 64.4 to 79.5% in E. maculatus. The moisture content in the testis was found to range from 72.10 to 79.35% in E. suratensis. A decrease in the moisture content was observed in the gonads of both species with the progress of maturation, which increases after spawning. Similar observation has been made in Clupea harengus by Iles (1984). Correlation studies showed that the moisture



content between muscle and ovary, muscle and testis and liver and testis are closely interrelated and fluctuate considerably in response to gonadal development in E. suratensis. However, no significant correlation was found between the moisture content of liver and ovary with response to gonadal maturation. In E. maculatus moisture content was observed to be associated between both ovary and muscle and ovary and liver.

### Ash

The ash content in the muscle and liver of E. suratensis was found to range between 2.62 to 4.42% and 2.51 to 4.29% respectively. In E. maculatus, the ash content in muscle and liver ranged between 2.75 to 4.8% and 2.91 to 4.6% respectively. These observations are comparable with those of the muscle and liver tissues of Dicentrarchus labrax (Stirling, 1976) mullets (Narain and Erasmus, 1977) and H. fossilis (Chreni, 1980). Ash content in ovary of E. suratensis and E. maculatus ranged between 2.98 to 4.75% and 2.91 to 5.0% respectively, whereas ash contents of testis ranged from 2.02 to 3.37%. A decrease in ash content was observed in muscle and liver of E. suratensis and E. maculatus as the maturation progressed, which increased after spawning. Contrary to this, an increase in ash content was observed in gonads during the gonadal development which decreased after spawning in both species. Chreni (1980) reported that the maximum ash values in muscle and liver of H. fossilis are associated with the ripening and ripe stages

in gonad maturation cycle. She correlated higher ash values during maturation with enhanced mineral metabolism of H. fossilis. In the present study, the ash content was low in muscle and liver and high in gonads of matured fishes.

Statistical analysis showed that the ash content translocation is more related to muscle and testis, muscle and ovary and liver and ovary than liver and testis in E. suratensis, while in E. maculatus it was closely related to muscle and ovary than to liver and ovary.

#### Lipid

The lipid content of the blood serum of E. suratensis is comparable with that of Salmo gairdneri (Takashina et al., 1972) and Platyichthys flesus (Peterson and Emmerson, 1977). However, it is higher than that shown by Varghese (1983) in the same species. Differences in lipid content in serum have also been observed during the present study. Phospholipid is mobilised for egg production. Lipid phosphorus of serum of Salmo trutta showed a sharp increase during gonad build-up and a drop to lower values after spawning. The changes in the males follow the same pattern, but are less pronounced than those in the female (Love, 1970). Similar observations have been made in the flounder Platyichthys flesus by Peterson and Emmerson (1977). In the present study, sharp decrease in lipid content of serum has been observed with the advancement in maturation in E. suratensis, which increased after spawning

in female, whereas, in males a sharp decrease was observed up to stage (III) and thereafter almost remained constant up to stage V (spent).

The lipid content of the white muscle of E. suratensis and E. maculatus are comparable with the lipid levels observed in the muscle of Oncorhynchus kisutch (Macleod et al., 1960), Mullus cephalus, Liza dumerili, L. richardsoni, L. tricuspidens (Marais and Erasmus, 1977); Heteropneustes fossilis (Shreni, 1980) and E. suratensis (Varghese, 1983).

The lipid level of liver of E. suratensis and E. maculatus showed ranges which are comparable with the results obtained in Salmo salar (Braekkan, 1956); Dicentrarchus labrax (Stirling, 1976); and H. fossilis (Shreni, 1980). Lipid content of testis and ovary of E. suratensis and ovary of E. maculatus are comparable with the lipid content of ovary and testis of Oncorhynchus nerka (Idler and Bitners, 1960).

The wider disparity in the fat of fishes in different months of the year is a matter of great economic and industrial importance. The lipid content in different species varies within wide limits. Rhinoptera sewalli (0.50%), Sillago sihama (0.60%) and Cirrhinus mrigala (0.80%) are supposed to have minimum lipid content, while Hilsa ilisha (19.4%), Milopia silondia (12.1%) and Pangasius pangasius (10.8%) have maximum lipid content (Anon, 1962).

Fattening, in most of the fishes, occurs during gonadal regression and after the sexual activities have been curtailed and thus fattening seems to be closely associated with gonadal cycle. The fat reserves are the main source of energy for the synthesis of generative tissue. Moreover, they are partially transferred to the gonads and are included as nutritive material in the yolk of the oocytes, acting as the principal source of endogenous food for the developing embryo. The level of fat reserves in the body and the rate of their utilization in the process of gonad maturation, therefore, largely determine the efficiency of spawning itself. (Shul'man, 1974).

Female Ladus morhua (Gordyl, 1953) and Lamprosetta fluviatilis (Bentley and Pollett, 1965) contain relatively less lipid than males in the liver at various stages of maturity. A similar condition is seen in the liver of L. suratensis in the present study.

Increase in lipid content has been observed in the eel Anguilla anguilla (Lovern, 1938); in Acipenser narkus (Idler and Bitners, 1960), in Labeo rohita (Banerjee and Bagchi, 1969) and in H. fossilis (Pandey et al., 1976). Shul'man (1974) demonstrated in the Azov black sea fishes and Atlantic sardines that the decline in fatness is especially marked in the initial stage of ripening (stage II), when intensive stage of differentiation goes on in the gonads. The rate of fat expenditure drops with the onset of the period of major

growth of the oocytes (stage III and IV), when differentiation of the generative tissue has been basically completed and yolk is being accumulated in the oocytes. In E. suratensis and E. maculatus also, a decrease in lipid content of muscle and liver has been observed at the time of gonadal maturation, which increase after spawning. Contrary to this, an increase in lipid content was found in testis and ovary as the maturation progressed, which decreased after spawning. Hahn (1967) and Walker and Wilhoft (1970) have explained the decline in fat body size during reproduction as due to fat metabolism by high levels of steroids and hormones such as estrogen and testosterone. The steady decline of fat content in E. suratensis and E. maculatus can also be explained on the same basis. The clear correlation of the fat content with the gonadal cycle in these fishes also (E. suratensis and E. maculatus), is in agreement with the findings of Banerjee and Bagchi (1969) in Labao rohita and Pandey et al., (1976) in H. fossilis.

The maturation of salmonid species O. nerka results in an extensive depletion of the lipid reserves of the flesh. Not all the lipid is used for energy purposes; 8% of it is transferred to the female gonad and 0.5% to the male gonad during migration (Idler and Bitners, 1960). In Etroplus species also lipid content depletes during maturation. The fluctuation in the lipid content was found to be more associated with testis and liver and testis and serum than with testis and muscle in

male E. suratensis, while in the female, the correlation between ovary and muscle was found to be more significant than ovary and liver and ovary and serum, however, in both cases, there was a conspicuous drain on this energy resource in relation to the reproductive cycle. A similar situation is also observed in E. maculatus.

### Protein

The protein content in the serum of E. suratensis ranged between 2.69 to 4.62 gm/100 ml which is comparable with the observation of Varghese (1983) on the same species (2.62 to 3.04 gms/100 ml ). Present observation on the serum protein level also comes in the range reported in Oncorhynchus kisutch (MacLeod et al., 1960) and Salmo gairdner (Alexander, 1977). Differences in the serum protein levels have been reported in male and female Salmo gairdner (Alexander, 1977). In the present investigation also difference in the serum protein content in male and female has been noticed in E. suratensis.

Several workers have shown that the concentration of proteins in the blood is always greater in the females during the spawning period (not at other times) (Love, 1960, Idler and Bitners, 1960). Similar results were also observed in E. suratensis in the present study. Love (1960) suggested that this can be satisfactorily explained as protein in transit from other organs to the larger gonads of the female. Bailey (1957) reported a rise in the blood calcium of the

females and suggested that this represents the mobilization of the protein ovovitellin, which has calcium linked to the molecule from the liver.

Philip et al., (1964) reported that serum protein content in Salvelinus fontinalis shows a rise during breeding season, more prominent in females that declines after spawning. In the present study, progressive decrease was found in the serum protein level of male E. suratensis during gonadal development, which increased slightly after spawning, while in female the protein content of serum showed decrease in stage II and increase in stage III followed by a decrease in stages IV and V. It is assumed that the protein formed in the liver and released into the blood, gets transported to the ovaries to be deposited in the developing eggs as suggested by Philip et al., (1964).

The protein content of the muscle of E. suratensis and E. maculatus are comparable with the protein levels of muscle of E. kisutch (MacLeod et al., 1960), Macil cephalus, Liza dumerili, L. richardsoni, L. tricuspidens (Marais and Erasmus, 1977) H. fossilis (Shreni, 1980). However, these levels were higher than those reported by Varghese (1983) on the same species.

The protein content observed in liver of E. suratensis and E. maculatus is comparable with the protein content

reported in liver of Clupea harengus, Gadus virens, Hippoclossus hippoclossus, E. salar (Brækken, 1959), O. kisutch (MacLeod et al., 1960), H. fossilis (Shreni, 1980) E. suratensis (Varghese, 1983). Protein levels of testis and ovary of E. suratensis and ovary of E. maculatus are comparable with the protein level of E. denisonius (Shivakani, 1980).

Females of Salmo salar (Belding, 1934), Gadus morhua (Koordyl, 1953) and Tinca tinca (Ziecik and Shawinski, 1965) were reported to contain relatively less protein in the muscle at various stages of maturity. Present investigation also is in full agreement with the earlier findings. Protein content has been reported in various fishes to decrease with the advancement of maturation followed by an increase after spawning (Bruce, 1924; Lovern and Wood, 1937; Koordyl, 1953; Danberg, 1963; Tripathi and Meur, 1972; Masurekar and Pai, 1979; Ochiai and Tanaka, 1980 and Iles, 1984). In E. suratensis and E. maculatus, a decrease in protein content of muscle and liver was found during gonadal development which increased after spawning. Contrary to this, an increase in protein content of testis and ovary was observed with the advancement of maturation followed by a decrease after spawning, indicating a clear case of translocation of the protein resources from somatic tissues to the gonads. Similar conditions was noticed in the developing ovaries of Gadus morhua by Brækken (1958).



Love (1960, 1962) stated that the build up of gonads is usually accomplished at the expense of body proteins. Dietary proteins seem to be inadequate to satisfy the huge demands made by the sex organs while eggs and sperms are maturing. In non-fatty fish, the whole process may be observed by means of water determination. As protein is removed, the water content rises steadily and is therefore a useful index of depletion of the fish. In the present study also the protein content in muscle and liver was observed to be decreasing, whereas water content was increasing with gonadal maturation in both E. suratensis and in E. maculatus. Fluctuations in the protein content with respect to maturation was found to be significant between testis and serum as compared to that between muscle and testis and liver and testis in male E. suratensis, whereas in female the correlation is more significant between muscle and ovary and serum and ovary than liver and ovary.

In the female of E. maculatus, the reproductive drain of the protein content from the liver to the ovary seems to be more pronounced than that from the muscle to the ovary.

### Glucose

The serum glucose level in Fundulus heteroclitus (Leach and Taylor, 1976), Spicara cyrillia (Fernandes and Planas, 1990) and in E. suratensis (Varghese, 1983) have been reported to

range from 42.0 to 144.7 mg/100 ml. In the present study also, the glucose content (50.26-108.44 mg/100 ml) has been found to be well within those ranges of other species. Like in E. heteroclitus; in E. suratensis also, the difference in glucose content in male and female specimens has been observed.

In Myxine glutinosa no difference was detected between the blood glucose contents of egg bearing and non-egg bearing females (Solkner and Winblad, 1964). Nace et al., (1964) and Robertson et al., (1961) observed a rise in the blood sugar of Opsanus tau and Onchorhynchus tshawytscha, respectively during maturation of gonads, but in Lamprolaima fluviatilis (Bentley and Follett, 1965) and in O. narka (Jones and MacLeod, 1960) there was a moderate fall with little difference between the sexes in the latter species. In the present investigation also, a sharp increase in serum glucose level was observed with the advancement of maturation in both male as well as female E. suratensis. Difference in serum glucose level between the sexes has also been observed, which agrees with findings of blood glucose levels in other species.

Although, it has been reported that glucose may be present as free sugar in the muscle from freshly killed fish, it has been suggested that sugar may be the product of early postmortem glycolysis and not necessarily present in the living tissue in significant amounts (Love, 1970).

The carbohydrate contents of muscle of male and female E. suratensis and E. maculatus female are comparable with the carbohydrate levels reported in muscle of L. labrax (Stirling, 1976). whereas, the carbohydrate content of liver are comparable with the carbohydrate level of liver of E. suratensis reported by Varghese (1983). The carbohydrate contents of ovary and testis could not be compared due to the non availability of the information.

An increase in the carbohydrate content was observed in blood serum, testis and ovary with the advancement in maturation followed by a decrease after spawning. Greene (1926) also reported increase in glucose during development, which is supported by the present findings in E. suratensis and E. maculatus. A decrease in carbohydrate level was observed in muscle and liver with the progress in maturation, which increased slightly after spawning. Translocation of carbohydrate in response to reproductive stress seems to be significantly correlated from muscle and liver to testis as compared to that from serum to testis in the male of E. suratensis, while in the female the correlation in respect of the carbohydrate levels between the serum and ovary is more significant as compared to that of muscle and liver with ovary.

In female, E. maculatus, the carbohydrate content is more involved with muscle and ovary and liver and ovary

for translocation during gonadal development.

A significant point to be mentioned here, is, that at the beginning of maturity, the level of protein, lipid, carbohydrate and moisture contents in muscle and liver are almost identical in both sexes. With progress in maturation, energy rich constituents such as protein, lipid and carbohydrate decrease in somatic tissues of both sexes recording the minimum at stage IV. Simultaneously, moisture content increases with the advancement of maturation in both males and females recording maximum in stage IV. In the spent stage, levels of protein, carbohydrate and lipid are consistently high and moisture content is consistently low, in both the sexes. In contrary to this, protein, lipid and carbohydrate content was observed to increase in testis and ovary upto stage IV, but decreases after spawning. This parallelism is suggestive of similar lines of biological course of translocation of biochemical constituents in both the sexes for the purpose of maturation and spawning. If the low values observed at stage I in both sexes are interpreted in terms of withdrawal for germ and oocyte production, then the increasing trend displayed at stage V (spent) can be interpreted as due to storage of constituents due to heavy feeding.

The moisture content of any organism can be expected to show variations consistent with the changes that occur in

the organism. These variations have some bearing on lipid variation also, so that in many instances, an inverse relationship between water and lipid contents has been recorded (Love, 1957; Iles and Wood, 1965; Yaganabano, 1977). The striking relationship between lipid and water manifested as an increase in the proportion of one leading to a decrease of the other, so that the total percentage of the two constituents is approximately constant, has been recorded by many workers (Iles and Wood, 1965; Love et al., 1972). Accumulated experimental evidences on E. auratensis and E. maculatus also show that water and lipid content are complimentary to each other. In the present study, protein, lipid and carbohydrate were observed to be inversely related to moisture in both species.

Iles (1984) established the relationship between the biochemical constituents of somatic tissue and gonads in Atlantic herring Clupea harengus. He reported that some time after feeding, protein metabolism begins and somatic growth is initiated as an anabolic process. The preparation of protein sub-units for subsequent gonad maturation continues, so that somatic growth and gonad growth differ only in the timing of use of material prepared at the same time by the same parental metabolic process (Iles, 1984).

Later in the season, there is a fairly short period over which the anabolic growth comes down to zero and translocation of protein to the gonad begins. This coincides with

a marked reduction in appetite resulting in the cessation of feeding (Iles, 1984). The final stage of maturation, certainly involves only the translocation of prepared materials.

In the present study also, protein, lipid and carbohydrate is found to decrease in muscle and liver with advancement of maturation followed by an increase after spawning in both E. suratanis and E. maculatus. Contrary to this, these biochemical constituents increases in gonads during gonadal development and decrease after spawning. It is suggested that the protein, lipid and carbohydrate are accumulated in immature stage in the muscle and liver which is later translocated to the gonads during gonadal development for sperm and oocyte formation. The present observations on E. suratanis and E. maculatus are in full agreement with the studies made by Iles (1984) on C. harengus.

## 6. BIOCHEMICAL GENETIC STUDIES ON ETROPLUS SURATENSIS AND ETROPLUS MACULATUS

### 6.1. Introduction

Genetic characters, showing qualitative variations between individuals, are of value for the differentiation and identification of sub-populations. The search for hereditary characters has largely been carried out by means of serological and biochemical methods.

Biochemical genetic studies have therefore, in recent times, gained widespread acceptance as a tool for stock identification, species separation and hybridization (Wishard *et al.*, 1980a and b). Apart from this the relationship between an organism's adaptive strategy in nature, genetic variability as measured by molecular studies and analysis of variance in quantitative traits are keys to the development of a theory that can predict the genetic adaptability of a species to aquaculture (Hedgecock *et al.*, 1976). Therefore, detailed information on the biochemical genetic characteristics of aquaculture species is important.

The analytical techniques of biochemical genetic studies (electrophoresis) are based on the distribution of simple genetic protein variants among individuals and populations. These variants which have been shown to be coded by single genes are inherited according to simple

genetic principles and remain unchanged throughout the life of the organism and are passed on from generation to generation (Utter et al., 1974).

A significant difference between biochemical and other more classical stock separation techniques such as colouration, meristic counts, growth rate and morphological measurements, is that the biochemical techniques detect only genetic differences. The classical parameters may reflect environmental parameters to an unknown extent, so that studies using controlled environments are necessary to establish the genetic basis of trait. Furthermore classical traits may be influenced by more than one gene or the interaction of several genes, with the result, the different genotypes may yield the same phenotype (Wishard et al., 1960 a, b.)

Biochemical genetic techniques detect the inter and intraspecific quantitative genetic variation. These techniques have effectively assisted in assessing either the efficiency or influence of artificial hybridization, gynogenesis and polyploidy. Gene environment interaction has also been detected by the application of biochemical genetic methods. Existence and mechanism of gene duplication have been determined by using biochemical genetic data in conjunction with chemical cytogenetic techniques (Utter et al., 1974). There has been a considerable amount of work on biochemical genetic



aspects of fishes. Proteins and isozymes have been used as markers.

Biochemical techniques designed to compare species on the basis of protein differences were started by Nuttal (1904) who used immunological methods to compare the serum of humans with that of other primates. Since then more refined techniques have led to better results at the protein level in taxonomy. The analysis of proteins is the simplest, most indirect approach to understanding the structure and function of the genetic material, deoxyribonucleic acid (DNA). Interest in these analyses arises because of the close relationship between protein structure and gene structure. Thus, by comparing the properties of homologous proteins from different taxa, one is in essence comparing their genes (Marmur *et al.*, 1963). It is now an established fact that genetic information coded in molecules of DNA is translated through a series of reactions in the structure of proteins which form the principal morphological units of the animal body at the molecular level of organization (Sibley, 1962).

A convenient method of comparing molecular differences between species is to measure the electrophoretic mobility of proteins by electrophoresis. Electrophoresis is a procedure that depends upon the movement of charged molecules in an electric field. The rate of movement depends on the voltage

applied and the overall electrical charge of each molecule. Protein molecules move in an electric field because they are composed of amino acids, many of which are charged.

Each tissue of fish contains several kinds of proteins, which form the basis for both the structure and metabolism of the tissue. Electrophoresis provides a means of separating protein molecules that have different electrical charges. Therefore, it can be used to determine the pattern of protein, characteristic of a species of fish (Shaklee and Salini, 1963).

Information from electrophoretic studies on proteins of muscle myogens, eye lens, blood serum and blood haemoglobin and their bearing on the taxonomy of E. suratensis and E. maculatus are presented in this study.

#### Muscle proteins (Myogens)

Connell (1953) and Dingle et al., (1955) who used the 'Tiselius' technique of electrophoresis were the first to analyse the muscle proteins of fishes for comparative purposes (Ramoir, 1955). Hewitt et al., (1963) studied the water soluble muscle proteins of 20 species of poeciliid fishes and found constant, marked and reproducible differences between various tribes and genera in the family. The electrophoretic muscle myogen patterns have been shown to be species specific (Uthe et al., 1966; Tsuyuki et al., 1965; Johnson et al., 1972

and Jamieson and Turner 1980) and quite independent of physiological factors such as sex, maturation and age (DeLigny, 1969; Utter et al., 1974; O'Rourke, 1974 and Turner and Groose, 1980). Interspecific hybridization experiments by Tsuyuki and Roberts (1965) supported the hereditary nature of these muscle constituents. Alteration in the electrophoretic protein patterns of refrigerated fish have been demonstrated by Moore et al., (1970). Adults and juveniles of grey mullets from the Mediterranean coast of Israel have been identified using protein patterns by Hersberg and Pastour (1975).

Intraspecific variations of muscle proteins were first reported by Hewitt et al., (1963) in the genus Mollienesia of the family Poeciliidae. Similar variations have also been reported in other fishes (Haen and O'Rourke, 1969) but these variations were not seen in specimens of approximately the same age in the fishes of genus Mollienesia (Hewitt et al., 1963).

Utne and Ryder (1970) reported regional variation in muscle myogen polymorphisms in Walleye (Stizostedion vitreum vitreum) collected from various Canadian lakes and Lake Superior.

#### Eye lens proteins

The soluble proteins of eye lens have great value in taxonomic studies, since the eye lens proteins are synthesized by only one cell type present in the eye as a single layer.

Eye lens nucleus is inert and more suitable for such study. Further they are very stable and can tolerate temperatures as high as 79°C before precipitating and can withstand lack of refrigeration up to 5 days without denaturing (Smith, 1970). They do not fluctuate physiologically, rhythmically or seasonally throughout the post embryonic life of the animal. In eye lens nucleus, though the proteins are not present in very high concentration (Yamada, 1966), are readily soluble in many media (Wood and Berger, 1961), are not contaminated with proteins from other tissues (eg. muscle, blood etc.) and are easily and clearly separable by electrophoresis.

Many studies on related species using electrophoretic separation of eye lens nucleus proteins, have utilized extracts of whole lenses. A common finding in such studies has been a similarity in the protein patterns in the categories of genus (Tsuyuki *et al.*, 1968, Menezes 1976 a), subfamily (Barret and Williams, 1967), family (Bon *et al.*, 1964; Maisel and Goodman, 1965) and class (Maisel and Goodman, 1965; Cobb *et al.*, 1968). Species specificity of electrophoretic patterns has also been described in members of the scombroid and corpaenoid families (Smith, 1970), in the *Archarhinids* (Peterson and Smith, 1969) and in the flat fishes (Menezes, 1976a), where proteins were extracted only from the nuclear portion of eye lens. Peterson and Shehadeh (1971) demonstrated the existence of sub-populations of Hawaiian mullet Mugil

Cephalus through eye lens protein studies. Smith (1971) used soluble eye lens nuclear proteins to demonstrate phylogenetic relationships.

The only report on the ontogenic variations in eye lens proteins of fishes is by O'Scarke, (1974) on species of trout. According to him, the eye lens nuclear proteins seem to offer an extremely useful clue to study the relationships regarding both higher taxa and interspecific differentiations. All the workers have so far unanimously supported the reliability of eye lens nucleus proteins in ichthyotaxonomy. Very recently the studies on 13 species of grey mullets by Reddy (1977) indicated reliability of eye lens nuclear protein patterns in ichthyotaxonomy.

#### Serum proteins

Sera from fishes have been extensively studied since the beginning of this century and Brooke (1964) has given a good review of this work. Species specificity of blood serum proteins has been shown by various workers (Moore, 1945; Irisawa and Irisawa, 1954; Tsuyuki and Roberts, 1965; Badawi and Said, 1971; Avtalion *et al.*, 1975, 1976; Meneses, 1976a,b).

Sex and age differences in serum proteins, first reported by Drilhon (1954) in carp were confirmed later in other species by other workers (Yamashita, 1968 a,b; 1969; Pesch, 1970; Gray and Mckensie, 1970, and Aida *et al.*, 1978).

Seasonal variations were also reported by Saito (1957) and changes affecting the serum due to disease have also been reported in many fishes (Phillips *et al.*, 1957; Mulcahy, 1967; Pesch 1970). Changes in serum protein patterns have also been reported as response to environmental factors (Fujiya, 1961; Thurston, 1967).

Maturation causes complex changes in the blood proteins. Pattern differences associated with maturation have been shown by many workers (Pesch, 1970; Utter *et al.*, 1974; Plack and Fraser, 1970; Aida *et al.*, 1973; Kirsipuu, 1975). Kirsipuu, (1975) also observed that some glycoprotein fractions do not vary with the sex of the fish or the season.

### Haemoglobin

Many investigators using electrophoresis to study fish haemoglobins have revealed a greater multiplicity of haemoglobins in teleost fishes than in other vertebrate groups. These haemoglobin molecules are formed by the association, into biquaternary molecules of globin chains coded by distinct gene loci (Manwell and Baker, 1970).

Electrophoretic studies on fish haemoglobins (Hbs) were first published in 1959 by workers in America, India and Japan (Behler and Shanks 1959; Chandrashekar, 1959 and Hoshimoto and Matsuura (1959).

Hoshimoto and Matsuura (1969a, b) later reported on its heterogeneity and species specificity. Sick (1961) was the

first to demonstrate intraspecific and geographical variations in Hb in Gadus morhua and explained these variations as reflections of gene frequency.

Interspecies differences have been reported in a number of fishes by various workers (Tsuyuki et al., 1965; Tsuyuki et al., 1968; Tsuyuki and Westrheim, 1970; Chen and Tsuyuki, 1970; Hines et al., 1971). Developmental variations which often continue throughout life have been demonstrated in the haemoglobin patterns of certain clupeoids, most salmonids and at least two cyprinid fishes (Koch et al., 1964; Vanstone et al., 1964; Wilkins and Iles, 1966; Perez and Maclean, 1976; Cross and O'Rourke, 1978).

Allelic variations occur at globulin gene loci in many fish species and these gene frequency differences have been used in stock identification, notably in cod, Gadus morhua (De Ligny 1969). A number of groups (Sick et al., 1973; Aspinwall and Tsuyuki, 1968; Chen and Tsuyuki, 1970; Hines et al., 1971; Cross and O'Rourke, 1978) have used electrophoresis to investigate the haemoglobin of hybrid fishes and have shown that, while the haemoglobin patterns of certain first generation (F 1) hybrids were summation of parental patterns, others contained additional fractions formed by random association of parental globins.

### Isoenzymes

The term isoenzyme has been proposed by Markert and Moller (1959) to describe different proteins with similar enzymatic activity. However, the term isoenzyme is now officially recommended by the standing committee on Enzymes of the International Union of Biochemistry to describe the multiple molecular forms of an enzyme occurring in a single species. The changes in isoenzymic patterns are indicators of changes in gene expression and are reflections of genetic and epigenetic events inside and outside the cell.

Isoenzymes offer a number of important advantages over more conventional morphological markers. Isoenzyme variants frequently occur spontaneously and seldom produce obviously deleterious effects. Variant alleles are generally co-dominant making it possible to easily and positively identify heterozygotes as well homozygotes, and to monitor conveniently the time of the expression of the parental alleles in the heterozygotes. Since isoenzyme represent specific gene products, variants are more likely to represent single gene lesions than are complex morphological markers. Aside from the obvious utility of isoenzymes to the protein concerned with problems of enzyme structure and function, they have been used by geneticists to estimate the degree of genetic polymorphisms present in populations, genetic differences within and between species, differential gene expression during development, gene



dosage effects on enzyme structure and function, intra and intergenic complementation and heterosis and the evolution of genes and organisms.

Isoenzymes as biochemical genetic markers have found increasing use to obtain a better definition of the taxonomic rank of species and other taxons, as well as to analyse the phylogenetic relationships of related species and genera, and may well be suited for uncovering the phylogeny of closely related species (Scholl and Herzberg, 1972). Cumulative comparisons among loci between two taxonomic groups can be summarised by a variety of methods into indices of similarity, or conversely, genetic distance. Such studies have been conducted with fishes of various groups such as salmonids (Souck and Ball, 1968; Utter *et al.*, 1973, 1979), Walley (Clayton *et al.*, 1973), Snapper (Smith & Crossland, 1977) and flounder larvae and 0 group flounders (Smith *et al.*, 1980), *Lepomis* (Vowwyl, 1979); and Antarctic fish *Notothenia* spp. (Anderson, 1982).

Many species of fishes exhibit considerable phenotypic variability in body shape, size and colour. The genetic relationship between body forms within some of these variables in fishes have been investigated using isoenzymes as a marker (Smith and Robertson, 1981 and Smith *et al.*, 1983).

In view of the relative paucity of similar work for fish of the Indian waters, a study on biochemical genetics of the

species E. suratensis and E. maculatus was taken up. The expression of three groups of proteins (general proteins, lipo-proteins and glycoproteins) was studied in fish samples from Cochin and several other geographical locations in India. The protein patterns of fish from Cochin were analysed for variations in relation to size groups and to different maturity stages. Protein polymorphism was studied for both species in different geographical localities by scoring allelic frequency. In addition, six isoenzymes, alcohol dehydrogenase, lactate dehydrogenase, malate dehydrogenase, malic enzyme, esterase and acid phosphatase were studied and compared for their banding pattern in fish sample from Cochin. Of these six, three isoenzymes namely, lactate dehydrogenase, esterase and acid phosphatase were scored for allelic frequency. The above study was aimed at identifying the occurrence of populations and sub-populations of E. suratensis and E. maculatus in different geographical localities and within a single geographical locality, i.e., Cochin.

## 6.2. Methods

The samples of fish were analysed for the occurrence of

a) Proteins and b) Isoenzymes.

### a) Proteins:

Three groups of proteins, viz., general proteins, lipo-proteins and glycoproteins, were analysed for their occurrence/ expression. The analysis was carried out on samples obtained

from Cochin as well as on samples obtained from other identified geographical localities. While fish samples of all areas were studied for the phenomenon of genetic polymorphism by scoring allelic frequency, fish samples obtained from Cochin were further studied for variations in protein banding in relation to size, sex and maturity stages. Maturity stages were identified according to the scale described by Jayaprakash *et al.*, 1979 and Jayaprakash and Balakrishnan, 1981). Fish samples from Cochin were grouped into the following size groups for studying the expression of proteins:

	Size group	Length(mm)
<u>E. suratensis</u>	I	50-100
	II	101-150
	III	151-200
	IV	201-250
<u>E. maculatus</u>	I	30-45
	II	46-60
	III	61-75
	IV	76-90

Six tissues of E. suratensis were analysed for the expression of proteins, viz., muscle, liver, eye lens, ovary, blood serum and haemoglobin. In E. maculatus, four tissues, viz., muscle, liver, eye lens and ovary were analysed for the expression of proteins.

b) Isoenzymes

The following 6 isoenzymes were examined for their occurrence and expression in various tissues of both E. suratensis and E. maculatus.

<u>Enzyme</u>	<u>Abbr.</u>	<u>Code No.</u>
1. Alcohol dehydrogenase	Adh	1.1.1.1
2. Lactate dehydrogenase	Ldh	1.1.1.27
3. Malate dehydrogenase	Mdh	1.1.1.37
4. Malic enzyme	Me	1.1.1.40
5. Esterase	Est	3.1.1.1.
6. Acid phosphatase	Acph	3.3.3.2

A number of tissues were analysed for the expression of these isoenzymes in E. suratensis namely, muscle, liver, ovary, testis, spleen, kidney, heart, brain, intestine, stomach, gills and blood serum. In E. maculatus, tissues examined were muscle, liver, ovary, testis, spleen, kidney, heart, brain and intestines.

From the above six isoenzymes, the expression of lactate dehydrogenase, esterase and acid phosphatase were employed in eye and liver for scoring allele frequency and for further polymorphism studies.

Electrophoretic methodsEquipment

A cylindrical tank with facility to run 12 tubes at a time was used. It consists of an upper and a lower unit

made entirely of perspex except for the electrodes and glass gel tubes. The lower unit holds about 300 ml buffer and has a ledge on which the electrode holder is fixed. The upper buffer reservoir is punched and silicone rubber grommets are inserted to hold the gel tubes. Unused holes are closed with rubber stoppers. The upper unit is gently lowered so that each gel tube slips through a corresponding hold of the electrode holder in to the lower buffer solution and rests in position in three perspex insets. About 250 ml. buffer is added to the upper reservoir and the upper electrode unit rests on the reservoir vessel on its edges. Both electrodes are circular, and hence equidistant from every gel tube and sit submerged in buffer.

Electric current was supplied through a power pack having capacity to supply current of 0-100 mA and 0-500 volts.

#### PROSEDURE

The live fishes were stunned by pithing and the muscle, liver, eye, ovary, testis, spleen, kidney, heart, brain, intestine, stomach and gills were removed. Care was taken to see that no contaminating tissue was included. Before mincing the tissue with scissors, they were washed with normal saline water.

Tissues were homogenised individually in glass homogenisers using a suitable extraction medium. This was carried

cut in a thermocole box containing ice. The homogenized material was then centrifuged prior to analysis. Preliminary investigations using various speeds had already revealed that centrifugation at different speeds had no effect on the resolution of bands during electrophoresis. Proportion of tissues to extraction medium was 1:10 for protein analysis and 1:2 for isoenzyme analysis.

#### Standardization for protein analysis

#### Standardization of extraction

Tissues were extracted in four different media (Table 36). Best resolution with maximum number of bands was obtained in double distilled water. All bands resolved using double distilled water were clear, distinct and adequately stained. Therefore, double distilled water was used for further experiments to extract proteins from the tissues.

#### Quantity of sample

Five different quantities (10, 20, 30, 40 and 50  $\mu$ l) of muscle, liver, eye lens, ovary, blood serum and blood haemoglobin were used to resolve the bands. Optimum quantity of extract medium was 10  $\mu$ l for blood Hb, 20  $\mu$ l for muscle, eye lens, ovary and blood serum and 30  $\mu$ l for liver. (Table 3). For all further studies, 20  $\mu$ l of sample was used since it was the best quantity for most of the tissues to resolve clear distinct and maximum number of bands.

TABLE - 36 EFFECT OF DIFFERENT SOLVENTS ON RESOLUTION OF MUSCLE AND LIVER  
PROTEIN FRACTIONS OF ETROPLUS SURATENSIS

Sl. No.	Tissue	Extractants	pH	No.of bands	Separation	Distinction of bands
1	MUSCLE	a. Double distilled water	7.0	10	+	+
		b. Tris-HCl buffer (0.05M)	7.5	6	-	-
		c. Phosphate buffer	7.0	7	-	-
		d. Sucrose (0.07 M)	7.0	7	-	-
2	LIVER	a. Double distilled water	7.0	12	+	+
		b. Tris-HCl buffer (0.05)	7.5	10	-	-
		c. Phosphate buffer	7.0	9	-	-
		d. Sucrose (0.07 M)	7.0	9	-	-

\* Foot Note

Tissue M - Muscle L - Liver E - Eye

Separation + or Good : Bands were clear and well resolved

- or Poor : Bands were not clear and completely resolved

Distinction of bands

+ : Clear bands without any trailing or diffusion, sufficiently interspaced

- : Bands not clear, trailing on gels and no sufficient interspacing.

TABLE - 37 EFFECT OF DIFFERENT QUANTITIES OF SAMPLE ON RESOLUTION OF PROTEIN FRACTIONS OF DIFFERENT TISSUES OF E. SURATENEIS.

Sl. No.	Tissue	Quantity of tissue	No. of bands	Separation	Distinction of bands
1	MUSCLE	10 $\mu$ l	8	+	+
		20 $\mu$ l	10	+	+
		30 $\mu$ l	8	-	-
		40 $\mu$ l	8	-	-
		50 $\mu$ l	7	-	-
2	LIVER	10 $\mu$ l	11	+	+
		20 $\mu$ l	15	+	+
		30 $\mu$ l	19	+	+
		40 $\mu$ l	17	-	-
		50 $\mu$ l	11	-	-
3	EYE LENS	10 $\mu$ l	6	+	+
		20 $\mu$ l	7	+	+
		30 $\mu$ l	6	-	-
		40 $\mu$ l	6	-	-
		50 $\mu$ l	6	-	-



Sl. No.	Tissue	Quantity of tissue	No. of bands	Separation	Distinction of bands
4		10 $\mu$ l	14	+	+
		20 $\mu$ l	16	+	+
		30 $\mu$ l	14	-	-
		40 $\mu$ l	15	-	-
		50 $\mu$ l	12	-	-
5	LIVER SERUM	10 $\mu$ l	16	+	+
		20 $\mu$ l	17	+	+
		30 $\mu$ l	14	-	-
		40 $\mu$ l	12	-	-
		50 $\mu$ l	12	-	-
6	RABBIT HEMOGLOBIN	10 $\mu$ l	11	+	+
		20 $\mu$ l	10	-	-
		30 $\mu$ l	9	-	-
		40 $\mu$ l	8	-	-
		50 $\mu$ l	8	-	-

#### Small pore buffer pH

Muscle, liver, eye lens, ovary, blood serum and blood haemoglobin proteins were separated by using small pore buffer at five different pH levels (7.5, 8.0, 8.5, 8.9 and 9.5) in the running gel (Table 38). The best resolutions with maximum number of bands, sufficient interspacing and clarity of gels were obtained at pH 8.9 for muscle, liver, blood serum and ovary and at pH 8.5 for eye lens and blood haemoglobin proteins.

Since the best resolution was obtained for most of the tissues at pH 8.9, it was used for all further experiments to resolve the proteins.

#### Polyacrylamide gel concentration

Separation of muscle, liver, eye lens, ovary, blood serum and blood haemoglobin was done in five different concentrations (5.0, 6.3, 7.0, 7.7 and 10%) of acrylamide in running gels (Table 39). Optimum concentration for best resolution was 7.0% for all the tissues. For all further experiments 7.0% acrylamide concentration was used in running gel. Different gel concentrations were used following the method described by Subasini and Ravindranath (1981).

#### Staining and Destaining procedure

The following staining and destaining mixtures were tested using 7% gel to get the best resolution. Time variables

TABLE - 38 PROTEIN SEPARATION USING DIFFERENT ACRYLAMIDE GEL CONCENTRATION  
FOR BEST RESOLUTION

Sl. No.	Tissue	Number of bands				
		Acrylamide gel concentrations				
		5.0%	6.3%	7.0%	7.7%	10.0%
1	Muscle	8	9	10	9	8
2	Liver	7	10	18	7	4
3	Eye	6	4	7	7	6
4	Ovary	8	13	15	15	12
5	Blood serum	15	13	17	10	11
6	Blood haemoglobin	12	12	10	12	12

TABLE - 39 PROTEIN SEPARATION USING DIFFERENT pH OF SMALL PORE BUFFER  
IN RUNNING GEL FOR BEST RESOLUTION

Sl No.	Tissue	Number of bands				
		pH of small pore buffer				
		7.5	8.0	8.5	8.9	9.5
1	Muscle	7	8	8	10	7
2	Liver	5	4	12	18	9
3	Eyelens	6	10	10	7	6
4	Ovary	4	5	12	15	13
5	Blood serum	11	15	17	17	5
6	Blood haemoglobin	8	11	13	10	9

used for staining were 20, 30 and 40 minutes (Table 40).

#### Staining mixture

- a) Amido black (0.25%) Loba
- b) Coomassie brilliant blue GR-250 (0.25%) Loba
- c) Kenacid blue - R (0.25%) BDH England.

Stains were dissolved in a mixture of methanol: double distilled water : acetic acid in 5:5:1 ratio.

#### Destaining Mixture

- a) Methanol : double distilled water : acetic acid = 5:5:1  
time - 1 hour.
- b) Acetic acid solution (7%) time - 4 hours.

Kenacid blue -R gave the best resolution as can be seen from Table 40. Clear and distinct bands and gels with maximum stability were obtained by staining for 20 minutes in Kenacid blue R and subsequently destaining in 7.0% acetic acid.

Some protein bands disappeared due to destaining for a long time in the gels which were stained by amido black and coomassie brilliant blue. Heavy trailing was observed in the gels stained with these stains. Destaining in methanol : double distilled water : acetic acid mixture resulted in shrinkage of gels.

TABLE - 40 MUSCLE PROTEIN SEPARATIONS IN DIFFERENT STAINING SOLUTION.

Sl. No.	Staining Mixture	Time of staining	No. of bands	Distinction of bands	Remarks
1	Amido black (0.25%)	20 minutes	8	Lightly stained	Heavy trailing on gels stains unstable and prolonged preserving in destaining solution results in loss of bands
		30 minutes	8	Moderately stained	
		40 minutes	8	Highly stained	
2	Coomassie brilliant blue GR-250 (0.25%)	20 minutes	10	Lightly stained	Trailing on gel stable stain
		30 minutes	10	Moderately stained	
		40 minutes	10	Highly stained	
3	Kenacid blue-R (0.25%)	20 minutes	10	Lightly stained	All bands clear and distinct, stain very stable
		30 minutes	10	Moderately stained	
		40 minutes	10	Highly stained	

All the stains were dissolved in a mixture of water: Methanol: Chloroform (3:3:1) solution. This was also used as destaining solution.

Kenacid blue - R for staining and 7% acetic acid for destaining and preservation were used in all further experiments.

#### Storage

Storage of sample extract in refrigerator at  $-4^{\circ}\text{C}$  had a marked effect on the tissue proteins. Increasing periods of storage resulted in less number of bands (Table 41). No change was observed when stored for 48 hours at  $-4^{\circ}\text{C}$ . Therefore all tissues were analysed within 24 hours of extraction from fresh tissues in all experiments.

#### Standardization of Enzyme analysis

##### Standardization of extraction

Four different media were used to extract lactate dehydrogenase from eye tissues (Table 42). But resolution with clear and distinct bands were obtained in double distilled water. Therefore, double distilled water was used in all further experiments to resolve the enzymes from all the tissues.

##### Quantity of sample and polyacrylamide gel concentration

Standard quantity of sample and polyacrylamide gel concentration used for isoenzyme separation was the same as used for protein separation.

##### Continuous and discontinuous buffers

To get the best resolution for different enzymes, various continuous and discontinuous buffers (Table 43) were used in

TABLE - 41 STORAGE EFFECT ON THE MUSCLE TISSUE PROTEIN FRACTIONS IN E. SUBATENSIS.

Period of storage in days	Band numbers										Total No. of bands
	1	2	3	4	5	6	7	8	9	10	
0	+	+	+	+	+	+	+	+	+	+	10*
2	+	+	+	+	+	+	+	+	-	-	8
4	+	+	+	+	+	+	-	+	-	-	7
6	+	+	+	+	+	+	-	+	-	-	7
8	+	-	+	-	+	-	-	+	-	-	4
10	+	-	-	-	+	-	-	+	-	-	3

+ Present

- Absent



TABLE - 42 EFFECT OF DIFFERENT SOLVENTS ON RESOLUTION OF LACTATE DEHYDROGENASE  
IN EYE TISSUE OF ETROPLUS SURATENSIS

Sl. No.	Extractants	pH	No. of bands	Seperation	Distinction of bands
a	Double distilled water	7.0	5	+	+
b	Tris-Cl buffer (0.05 M)	7.5	5	+	-
c	Phosphate buffer	7.0	5	+	-
d	Sucrose 0.07 M	7.0	5	+	-

TABLE - 43 BUFFER (CONTINUOUS AND DISCONTINUOUS) USED FOR ISOCYME SEPARATION

Sl. No.	Buffer systems	electrode Components (per Litre)			Gel components(per Lit.)		References
		pH	Buffer		pH	Buffer	
1	Tris Boric EDTA	9.0	Tris 10.53 g	Boric Acid 0.54 g EDTA 0.3 g	9.0	Dilute electrode buffer 1:10 with double distilled water	Ayala et al. (1972)
2	Tris-Glycine-HCl	8.4	Tris 6.0 g	Glycine 28.8 g	8.4	Tris HCl in dist- 36.6g 48 ml filled water up to 100ml	Davis (1964)
3	Tris-Citric-Boric LiOH	8.26	LiOH 2.51g	Boric Acid 18.54g	8.31	Tris Citric Electro- 3.63g acid de buffer 1.05g 10 ml	Ferguson and Wallace(1961)
4	Tris-Citric-Boric NaOH	8.10	Boric Acid 18.55g	NaOH 2.40 g	9.65	Tris Citric acid 9.20g 1.05g	Poulik(1957)
5	0.3 M Borate	8.0	Boric Acid 18.55 g	NaOH 2.0 g	8.5	Boric NaOH acid 0.48g 1.86g	Shaw and Prasad(1970)
6	0.5 M Tris Versene Borate	8.0	Tris 60.6g	Boric Acid 40.0g EDTA 6.0g	7.5	Tris Boric EDTA 6.06 6.00g 0.6g	Shaw and Prasad (1970)
7	0.155 M Tris-0.043 M citric Acid(Tris citrate)	7.0	Tris 16.35g	Citric Acid 9.04 g	7.0	Dilute 66.7 ml of electrode buffer to 1 litre	Shaw and Prasad (1970)
8	0.02 M Tris-0.01M Maleic-0.01M EDTA	7.4	Tris 12.1g	Maleic Acid 11.6 g EDTA 3.72g MgCl <sub>2</sub> 2.03g	7.4	Dilute electrode buffer 1:10 times with double distilled water	Shaw and Prasad (1970) Modified.

0.23 ml TMED( N<sub>1</sub> N<sub>1</sub> N<sub>1</sub> N<sub>1</sub> - Tetramethylethylene diamine) was added in 100 ml of all buffers.

gels and electrodes. Muscle, liver and eye tissues were tested for Ldh, Est, and Acph in each buffer system. The best resolution of Ldh and Est with maximum number of clear, distinct and sufficiently interspaced bands were obtained in Tris-HCl-Glycine buffer pH 8-9/8.4 and of Acph in Tris-maleic-EDTA buffer pH 7.4. Buffer Tris-HCl Glycine also resulted in best resolution for Adh, Pdh, and Me enzymes. A comparison of Ldh, Est and Acph resolved in different buffer systems are presented in Tables 44, 45 and 46 respectively.

#### Staining and Destaining

Staining of all the enzymes were done by the methods described by Redfield and Salini (1980). Destaining solution used for enzyme was 7% acetic acid.

#### Genetic polymorphism studies

The following numbers of individuals were examined for the occurrence of the enzymes; Ldh, Acph and Est in E. surattensis and E. maculatus. All individuals were assumed to have been taken from the same random mating population.

<u>Species</u>	<u>Enzyme</u>	<u>No. of individuals</u>
<u>E. surattensis</u>	Lactate dehydrogenase	145
<u>E. maculatus</u>	"	183
<u>E. surattensis</u>	Acid phosphatase	152
<u>E. maculatus</u>	"	178
<u>E. surattensis</u>	Esterase	168
<u>E. maculatus</u>	"	177

TABLE - 44 LACTATE DEHYDROGENASE PATTERNS OF DIFFERENT TISSUES OF E. SURATENSIS RESOLVED  
IN DIFFERENT BUFFER SYSTEMS

No.	Buffer systems	Migration time	Tissue	No. of bands	Intensities of bands					Sepa- rat- ion	Inter- spac- ing	Disti- nction of bands
					1	2	3	4	5			
1	Tris- Boric- EDTA Fast 30 min pH 9.0		M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	1	XXXXX					-	-	+
2	Tris- Glycine - Slow 90 min CH1 pH 8.4		M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	5	XXXXX	XXXX	XX	XXX	XXXXX	+	+	+
3	Tris- citric - Fast 30 min Boric - LiOH pH 8.26		M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	1	XXXXX					-	-	+
4	Tris- citric- Moderate Boric- NaOH 60 min pH 8.10		M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	2	XXXXX	XXXXX				-	-	-

No.	Buffer systems	Migration time	Tissue	No. of bands	Intensities of Bands					Separation	Inter- representing	Dist- in- ction of bands
					1	2	3	4	5			
5	0.3M Borate pH 8.0	Slow 80 min	M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	1	XXXXX					-	-	+
6	0.5M Tris versene Borate pH 8.0	Slow 80 min	M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	5	XXXXX	XX	XXX	XX	XXXXX	+	-	+
7	0.155 M Tris 0.043 M Citric Acid(Tris-Citrate) pH 7.0	Fast 30 min	M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	5	XXXXX	XX	XXX	XX	XXX	+	-	+
8	0.02M Tris 0.01 M Maleic Acid 0.01 M pH 7.4	Fast 30 min	M	1	XXXXX					-	-	+
			L	1	XXXXX					-	-	+
			E	5	XXXXX	XX	XXX	XX	XXXXX	+	-	+

XXXX - Dark  
 XXX - Medium  
 XX - Light  
 X - Faint

TABLE - 45 ENZYME PATTERNS OF DIFFERENT TISSUES OF ETROPLUS SURATENSIS RESOLVED IN DIFFERENT BUFFER SYSTEM.

No.	Buffer system	Migration time	Tis- sue	No. of bands	Intensities of bands							Seps- rat- ion	Inte- rpa- cing	Dist- inct- ion of bands
					1	2	3	4	5	6	7			
1	Tris-Boric- NaOH pH 9.0	Fast 30 min	M	3	XXXX	XXXX	XXXX					-	-	+
			L	3	XXXXX	XXXXX	XXXXX					-	-	+
			E	2	XXXXX	XXXXX						-	-	+
2	Tris-Glycine-HCl pH 8.4	Slow 90 min	M	6	XXXXX	XXXXX	XXXXX	XXXXX	XXXX	XXXX		+	+	+
			L	7	XXXXX	XXXX	XXXXX	XXXX	XXXXX	XX	XXXXX	+	+	+
			E	5	XXXXX	XX	XXXXX	XX	XX			+	+	+
3	Tris-citric-Boric- NaOH pH 8.26	Fast 30 min	M	4	XXXXX	XXXXX	XXXX	XXXX				-	-	+
			L	1	XXXX							-	-	+
			E	5	XXXXX	XXXXX	XXXXX	XXXXX	XXXXX			+	-	+
4	Tris-Citric-Boric- NaOH pH 8.10	Moderate 60.0 min	M	4	XXXXX	XX	XXXX	XX				-	-	+
			L	4	XXXXX	XXXX	XXXX	XX				-	-	+
			E	4	XXXXX	XX	XXXXX	XX				-	-	+

Sl. No.	Buffer Systems	Migration/Time	Tis- sue	No. of bands	Intensities of bands							Sepa- ration	Inter- spacing	Dist- inct- ion of bands
					1	2	3	4	5	6	7			
5	0.03% Borate pH 8.0	Slow 80 min	M	4	XXXX	XXXX	XXXXX	XXXX				-	-	+
			L	2	XXXX	XXXX						-	-	+
			E	4	XXXX	XXXX	XXXX	XXXXX						
6	0.5 M Tris versene Borate pH 8.0	Slow 80 min	M	6	XXXXX	XXXXX	XXXXX	XXXXX	XXXX	XXXX		+	-	+
			L	4	XXXXX	XXXXX	XXXXX	XXXX				-	-	+
			E	2	XXXX	XXXXX						-	-	-
7	0.155 M Tris 0.043 Citric Acid (Tris citrate) pH 7.0	Fast 30 min	M	3	XXXX	XXXXX	XXXXX					-	-	-
			L	5	XXXXX	XXXXX	XXXXX	XXXXX	XX			-	-	+
			E	4	XXXXX	XXXXX	XXXXX	XXXXX				-	-	-
8	0.02 Tris 0.01 M Maleic Acid 0.01 M EDTA pH 7.4	Fast 30 min	M	4	XXXX	XXXX	XXXX	XXXX				+	+	+
			L	3	XXXXX	XXXXX	XXXXX					-	-	+
			E	2	XXXXX	XXXXX						-	-	+

TABLE - 46 ACID PHOSPHATASE PATTERNS OF DIFFERENT TISSUES OF L. M. PERCIN RESOLVED  
IN DIFFERENT BUFFER SYSTEMS

No.	Buffer systems	Migration time	Tissue	No. of bands	Intensities of bands		Sepa- rat- ion	Inter- spec- ing	Dist- inction of Bands
					1	2			
1	Tris-Boric-NaH <sub>2</sub> PO <sub>4</sub> pH 9.0	Fast 30 min	M	1	XXX		-	-	+
			L	2	XXX	XXXX	+	+	-
			E	2	XX		-	-	+
2	Tris-Glycine- HCl pH 8.4	Slow 90 min	M	1	XXX		-	-	+
			L	2	XXXX	XXXXX	+	+	-
			E	2	XX	XX	+	-	-
3	Tris-citric Boric-NaH <sub>2</sub> PO <sub>4</sub> pH 8.26	Fast 30 min	M	1	XX		-	-	+
			L	2	XXX	XXX	-	+	-
			E	2	XXX	XX	+	-	-
4	Tris-citric- Boric-NaH <sub>2</sub> PO <sub>4</sub> pH 8.10	Moderate 60 min	M	1	XXX		-	-	+
			L	2	XXX	XXXXX	+	+	-
			E	1	XXX		-	-	+



No.	Buffer Systems	Migration time	Tissue	No. of bands	Intensities of bands		separation	inter-spacing	Distinction of bands
					1	2			
5	0.3 M Borate pH 8.0	Slow 80 min	M	1	x		-	-	+
			L	2	x	XXXX	+	+	-
			E	2	x	XXX	+	-	+
6	0.5 M Tris ver- sene Borate pH 8.0	Slow 80 min	M	1	XX		-	-	+
			L	2	XX	XX	+	+	-
			E	2	XX	XX	+	-	+
7	0.155 M Tris 0.043 M Citric Acid (Tris cit- rate) pH 7.0	Moderate 60 min	M	2	XX	XX	+	-	-
			L	2	XX	XX	+	+	-
			E	2	XX	XX	+	-	-
8	0.02 M Tris - 0.01 M Maleic Acid 0.01 M EDTA pH 7.4	Fast 30 min	M	2	XXXX	XX	+	+	+
			L	2	XXXX	XXXX	+	+	+
			E	2	XXX	XXX	+	+	+

### Expression of protein bands

The protein bands were given serial numbers, the band closest to the cathodal region being No. 1 and increasing numbers assigned to the bands towards the anodal region. These bands were also grouped into three zones, I, II and III.

### Expression of enzyme variation

The designation for gene loci and allelic variants encoding the enzymes are as described by Ayala *et al.*, (1971). At each locus, one allele which is the most common is given the value 100. Other alleles are named with reference to that standard. An allele designated 0.95, codes for an enzyme which migrates 5 mm less than the standard, towards the anode and an allele coding for an enzyme whose migration is 5 mm more than the standard, is designated 1.05.

### Relative mobility value (Rf)

Rf value of each band was calculated as the ratio of the distance travelled by the band to that of the distance travelled by fastest moving band.

### Scoring allelic frequency

After obtaining the expression of a particular enzyme system and encoding the loci, a number of specimens were analysed for the same enzyme system in the same tissue for

any alleles if present at that loci and the frequency of that allele is estimated.

#### Estimation of genetic variation

The direct and most informative measure of genetic variation from gene frequency data is the average proportion of heterozygotes per locus (i.e., average heterozygosity). This quantity can be calculated by directly counting the proportion of heterozygotes observed or by using the expected "Hardy Weinberg" proportions and calculating the average proportion of heterozygotes using the observed gene frequencies.

The frequency of the allele was estimated for two allele system by the following equations:

$$P_A = \frac{2(AA) + (AB)}{2N} \text{ for (allele A)} \quad \dots \text{Eq. 1.}$$

$$Q_B = \frac{2(BB) + (AB)}{2N} \text{ for (allele B)} \quad \dots \text{Eq. 2.}$$

Similarly for 3 and 4 allele systems, allele frequencies were calculated using the modified formula based on the above mentioned formula.

#### Hardy Weinberg genetic model (Stern, 1941)

The observed allelic frequency was compared with expected frequency obtained using the Hardy Weinberg model. In the presence of two alleles

$$p^2 (AA) + 2 pq (AB) + q^2 (BB) = 1 \quad \dots \text{Eq. 3.}$$

Where p and q are the frequencies of alleles and AA, AB and BB are the genotypes of individuals when two alleles of one and the same locus are codominant. Here AA and BB are homozygous and AB heterozygous.

Similar calculations were made in the presence of three and four co-dominant alleles of one locus. Equations used were as follows:

For three co-dominant alleles

$$p^2 (AA) + q^2 (BB) + r^2 (CC) + 2 pq (AB) + 2 pr (AC) + 2 qr (BC) = 1 \quad \dots \text{Eq.4.}$$

For four co-dominant alleles

$$p^2 (AA) + q^2 (BB) + r^2 (CC) + s^2 (DD) + 2 pq (AB) + 2 pr (AC) + 2 ps (AD) + 2 qq (BC) + 2 qs (BD) + 2 rs (CD) = 1 \quad \dots \text{Eq.5.}$$

The difference between observed and expected values of allelic frequencies was tested with chi-square ( $\chi^2$ ) method.

$$\chi^2 = \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}} \quad \dots \text{Eq.6.}$$

A comparison between the number of observed heterozygotes ( $H_o$ ) and expected heterozygote ( $H_e$ ) was made using the relationship.

$$\frac{H_o - H_e}{H_e} = D \quad \dots \text{Eq. 7.}$$

### 6.3. Results of Biochemical Genetic studies

#### 6.3.1. Protein expression in tissues

##### Ectoparasitiscus auratensis

Qualitative changes in protein components of muscle, liver, eye lens, ovary, blood serum and blood haemoglobin have been determined to see whether age, sex or maturity stages exert any influence on the protein patterns of E. auratensis. The details of banding patterns, relative mobility values (Rf) and intensities of bands have been illustrated in plates III to VII and figures 12 to 19 and table 47.

##### Muscle

Study of muscle myogen patterns of E. auratensis reveals 10 bands, of which band No. 8 was the major common band with high intensity and density at 0.62 Rf value (Fig.12). Repeated experiments on muscle proteins from specimens of different sex and maturity stages as well as size groups, did not show any variation and produced the same patterns both in the number and electrophoretic mobilities of the bands. However, the staining intensities of the bands of Zone-I decreased with an increase in size but it did not vary between the sexes.

##### Liver

Comparative electrophoretic protein patterns of liver proteins in different size groups of E. auratensis showed 18

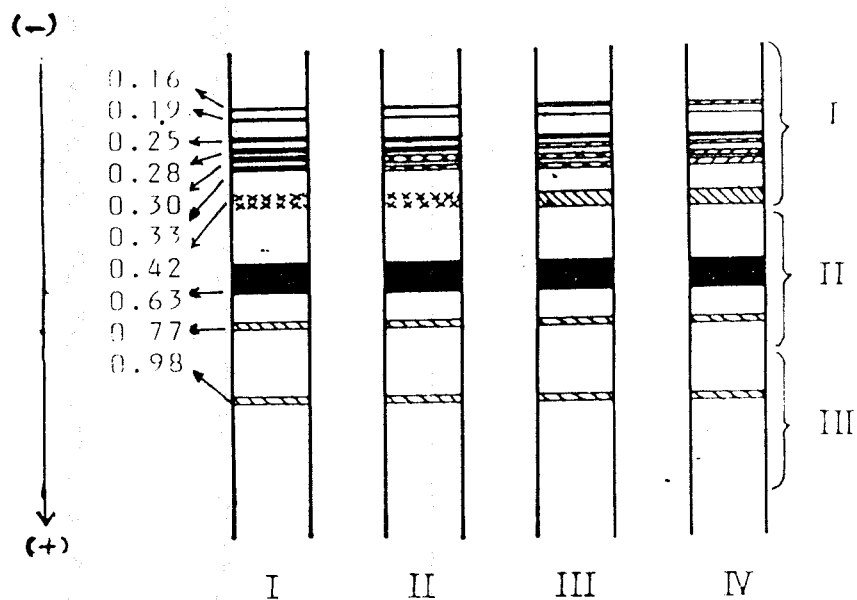
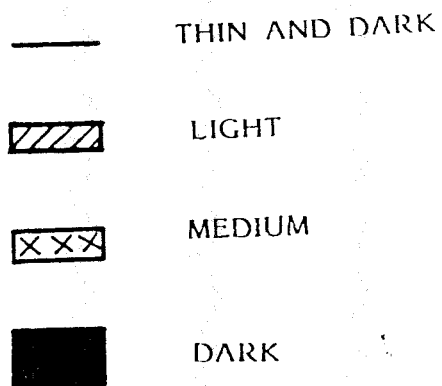


FIG. 12 COMPARATIVE ELECTROPHEROGRAMS OF MUSCLE PROTEIN PATTERNS IN DIFFERENT SIZE GROUPS OF ETROPLUS SURATENSIS

bands (Plate-IIIA, Fig. 13). Of these, band Nos. 6 and 12 were the major common bands in all size groups at Rf values 0.34 and 0.64 respectively. Size group I consisted of 14 bands, size group II of 17 bands, size group III of 15 bands and size group IV of 12 bands. Electrophoretic mobilities and intensities of the bands were identical between different size groups of fish. It was also observed that these patterns did not vary between the sexes of fish.

#### Eye lens

Protein patterns of eye lens of E. suratensis consisted of a total of 7 bands (Plate IIIB, Fig. 14). These patterns were consistent and did not show any variation due to age, sex or maturity stages.

#### Ovary

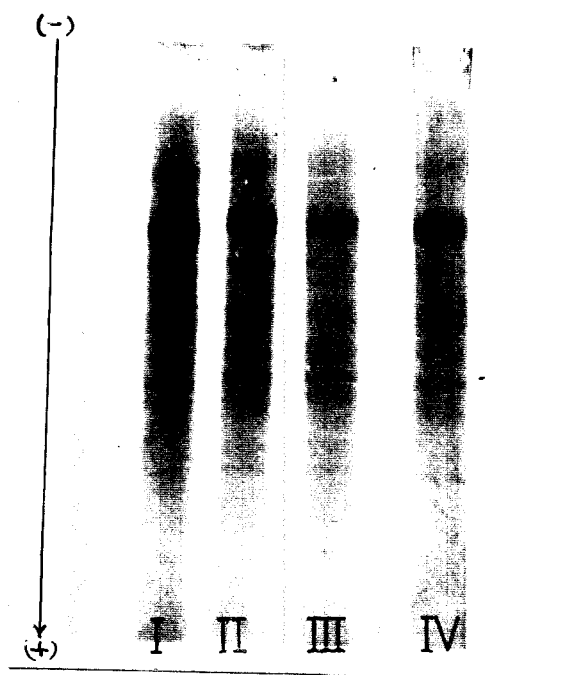
A comparison of protein patterns of ovary showed 16 bands at different stages of maturity. Close to the cathode, 4 thin bands were observed, which were grouped in the band system No.1 and considered to be the component bands of this system. The number of these component bands varied from 3-4 between different stages of maturity (Plate IV, Fig. 15).

Bands Nos. 2 and 4 were major common bands with maximum intensities in all stages. Rf value for band No.2 was 0.16 while it varied from 0.28 to 0.33 for band No.4 at different stages of maturity.

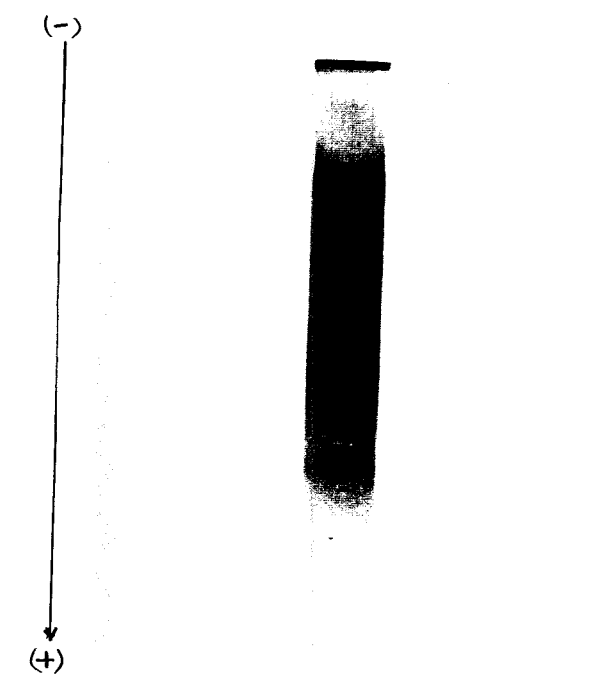
Plate IIIa. Electrophoretic patterns of liver proteins  
in different size groups of Etroplus suratensis.

Plate IIIb. Electrophoretic patterns of eyelens  
proteins of Etroplus suratensis.





A



B

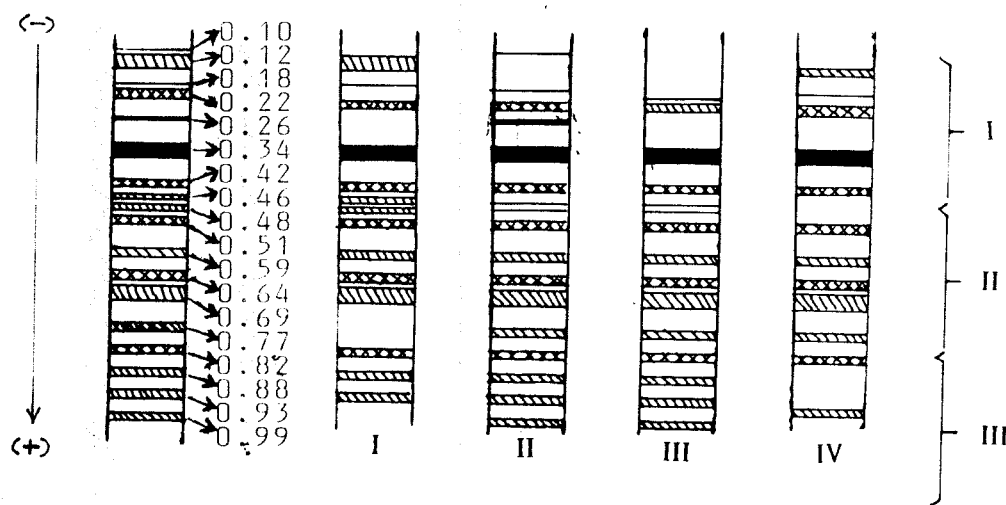


FIG. 13 COMPARATIVE ELECTROPHEROGRAMS OF LIVER PROTEIN PATTERNS IN DIFFERENT SIZE GROUPS OF ETROPLUS SURATENSIS

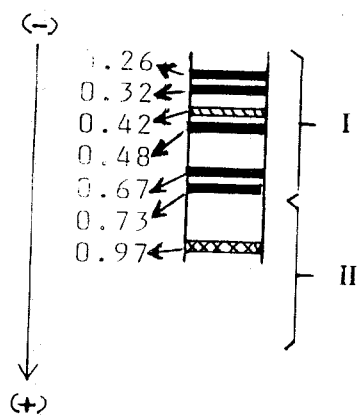
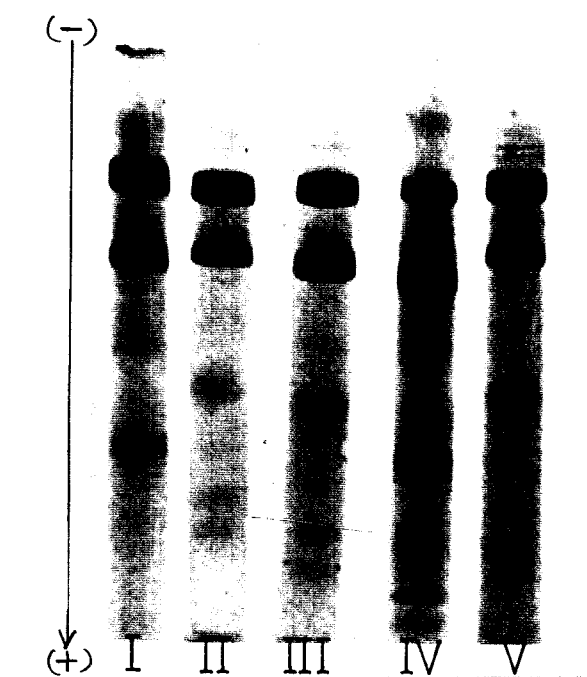


FIG. 14 ELECTROPHEROGRAM OF EYE LENS PROTEIN PATTERNS OF ETROPLUS SURATENSIS

Plate IV. Electrophoretic patterns of ovary proteins  
in different maturity stages of  
Etroplus suratensis.

PLATE IV



Ovary protein patterns consisted of 6 bands at maturity stage I, 7 at stage II, 8 at stage III, 13 at stage IV, and 7 at stage V. Electrophoretic mobilities of bands varied between different maturity stages of ovary.

#### Blood serum

Comparative blood serum protein patterns between sexes and at different maturity stages of female fishes are presented in plates V, a, V b and Figs. 16 a, 16 b.

It is evident that marked variation occurs between sexes and at different maturity stages of female fishes in serum protein patterns. Total of 15 bands were observed which had either common or different mobilities.

Serum protein patterns of male fishes consisted of 12 bands, which were similar to that of the first stage of female fishes in electrophoretic mobilities and staining intensities of the bands. However, patterns differed from II to IV stages of females. Repeated experiments produced consistent patterns and did not show any variation at different stages of maturity in male fishes.

A comparison of serum protein patterns at maturity stage IV of male and female fishes revealed that bands No.1, 3, 4, 5, 6, 8, 9, 11 and 13 were common in both sexes, but differed slightly in Rf values and staining intensities. In males band

Nos. 2, 7 and 14 and in females band Nos. 10, 12 and 15 were absent at the maturity stage IV.

Serum protein patterns of female fishes consisted 12 band systems at stages I, II, III and IV. In all stages of males and first stage of females band systems 1, 10, 11, 13 and 15 appeared as two component bands. Like-wise in female stage II band system 4, 13 and 15 and in stage III band system, 13 appeared as two component bands. In female stage II bands No.11 and 12 appeared as a single band.

#### Blood Haemoglobin

A comparison of blood haemoglobin patterns in different size groups of E. suratensis are presented in plate-VI and Fig. 17. Study reveals the presence of total of 13 bands. Of these 6 bands were observed in size group I. 7 bands were observed in size group I, 7 bands in size group II, 10 bands in size group III and 5 bands in size group IV. Band No.4 was the major common band with maximum staining intensity and Rf value 0.35 in all size groups. Intensities and Rf values of other bands varied between different size groups. No variations were observed in haemoglobin patterns either due to sex or maturity stages.

Plate VI. Electrophoretic patterns of blood haemoglobin  
in different size groups of E. suratensis.

PLATE VI





**Comparative protein patterns in different tissues of E. suratensis.**

Electrophoretic protein patterns of muscle, liver, eye lens, ovary, blood serum and blood haemoglobin showed considerable variation in the electrophoretic mobilities, staining intensities and number of bands (Plate VII, Fig. 18, Table 47). No common bands were observed between the tissues. Except in eye lens, protein bands were represented in all the three zones in other tissues. Eye lens proteins were limited upto zones I and II only.

Number of bands observed in different tissues and in different zones were as follows:-

	Zone I	Zone II	Zone III
Muscle	7	2	1
Liver	5	7	5
Eye lens	4	3	-
Ovary	*2	6	5
Blood serum	7	6	4
Blood Haemoglobin	4	4	2

\* Includes 4 component bands of band No.1.

**General, Lipo and Glyco-protein patterns**

Comparative electrophoretic protein patterns of general protein, lipoprotein and glycoproteins of muscle, liver, eye

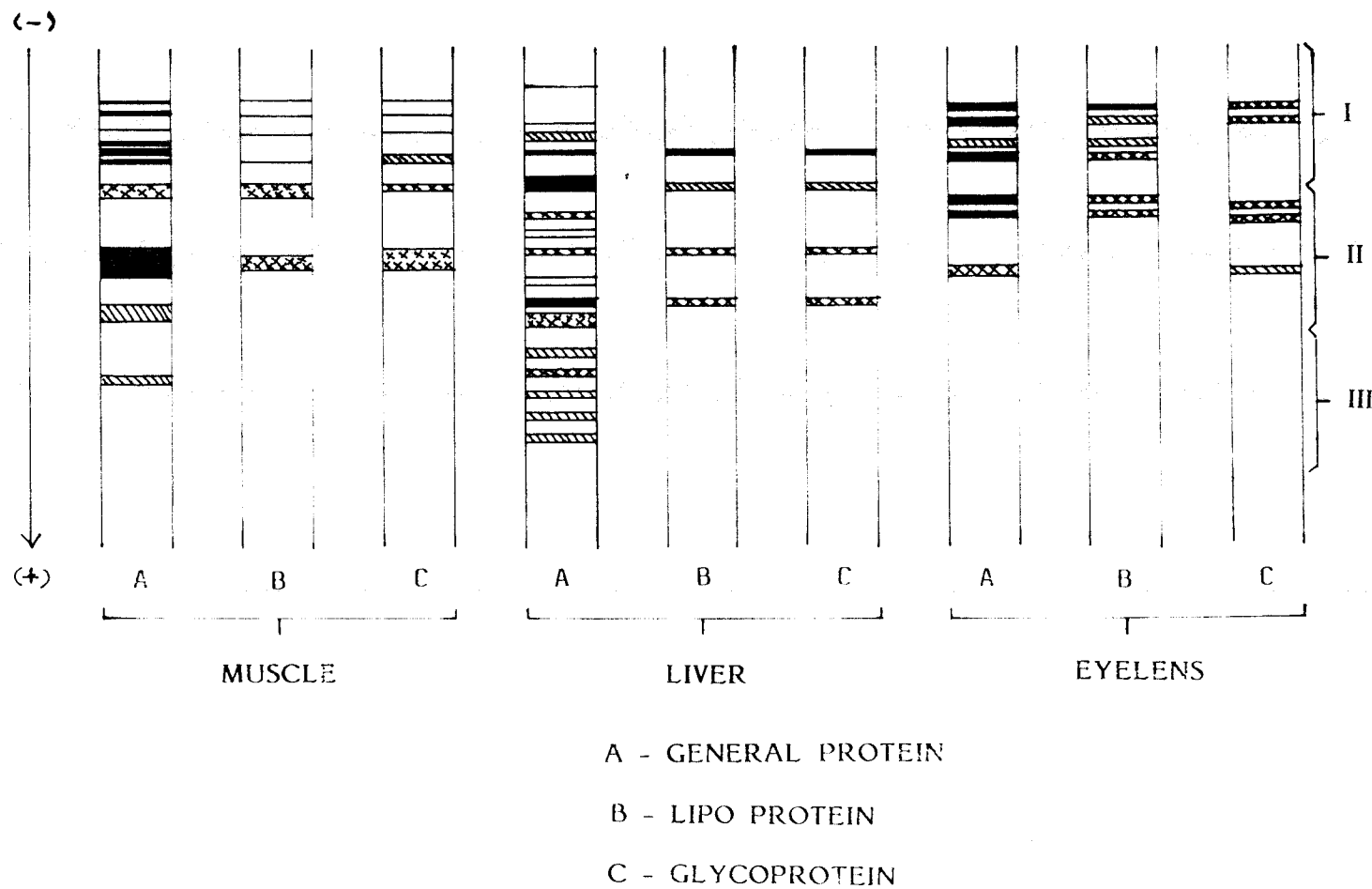


FIG. 19a COMPARATIVE ELECTROPHEROGRAMS OF GENERAL, LIPO AND GLYCO-PROTEIN PATTERNS OF MUSCLE, LIVER AND EYE LENS OF ETROPLUS SURATENSIS

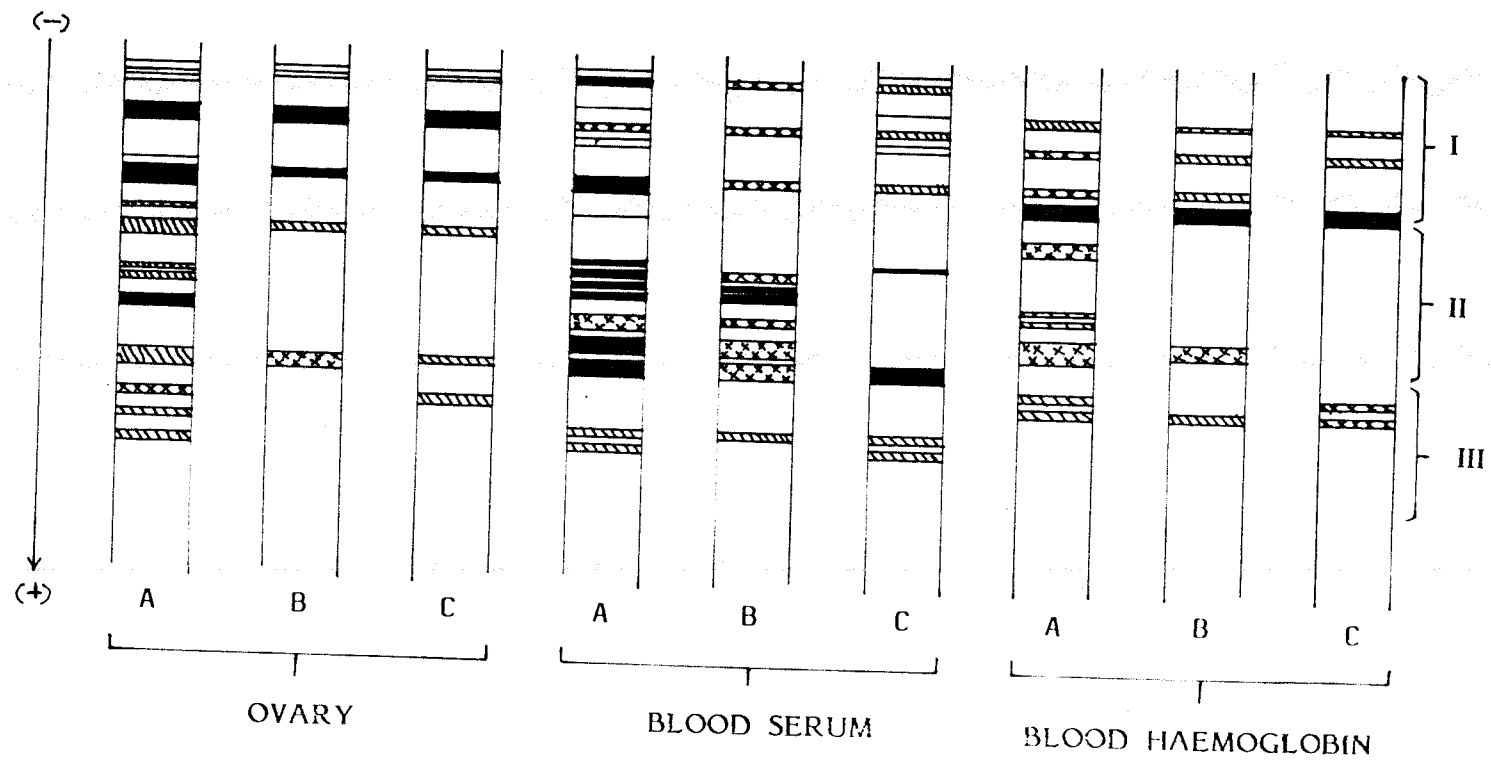


FIG. 19b. COMPARATIVE ELECTROPHEROGRAMS OF GENERAL, LIPO AND GLYCO-PROTEIN PATTERNS OF OVARY, BLOOD SERUM AND BLOOD HAEMOGLOBIN OF ETROPLUS SURATENSIS

lens, blood serum and blood haemoglobin are presented in Fig. 19. It was observed that the fractions obtained for lipo and glyco-proteins had the same mobilities as in general protein fractions. Muscle tissue consisted of 6, liver 4, eye lens 6, ovary 5, blood serum 10 and blood haemoglobin 6 lipo protein fractions. Similarly muscle consisted of 6, liver 4, eye lens 6, ovary 6, blood serum 11 and blood haemoglobin 5 glycoprotein fractions.

In muscle, liver and eye lens, glyco and lipo-protein fractions were in zone I and zone II, while in ovary, blood serum and blood haemoglobin these protein fractions were found in all the three zones.

#### *Etmopterus maculatus*

Electrophoretic analysis of muscle, liver, eye lens and ovary protein was done and results are presented in plates VIII to X figures 20-25 and table 43.

#### Muscle

The protein in the muscle tissue produced the same patterns consistently and did not show any variation either due to sex or maturity stages (Fig. 20). Intensities of the bands decrease as the fish grows.

The number of bands observed were 10. Band Nos. 1, 2, 5, 7 and 8 were the major common bands with maximum intensities at  $R_f$  values of 0.04, 0.18, 0.38, 0.66 and 0.77 respectively.

Rf value of each band was similar in different size groups of the fish.

### Liver

Studies on liver protein patterns revealed 10 bands. Band Nos. 5 and 6 were the major common bands in zone II a with maximum intensities at Rf values of 0.45 and 0.49-0.50 respectively. Size groups I and II consisted 11 bands, while III and IV consisted 10 bands. Intensities of bands decreased as the fish grew, however, Rf values of all the bands remained the same (Plate VIIIA, Fig. 21).

No variations were observed in the different sexes and maturity stages of fish.

### Eye lens

Repeated experiments on eye lens protein showed consistent patterns and did not show any variation due to age, sex or maturity stages. Studies revealed 8 protein bands. Band Nos. 1, 2, 3 and 7 were the major common bands, with maximum intensities at Rf values of 0.25, 0.41, 0.48 and 0.76 respectively (Plate VIII B, Fig. 22).

### Ovary

Considerable variations were observed in the protein fractions at different stages of ovary development. A comparison of electrophoretic patterns revealed total 10 band systems. Band system 1 had 4 component bands, out of which 2

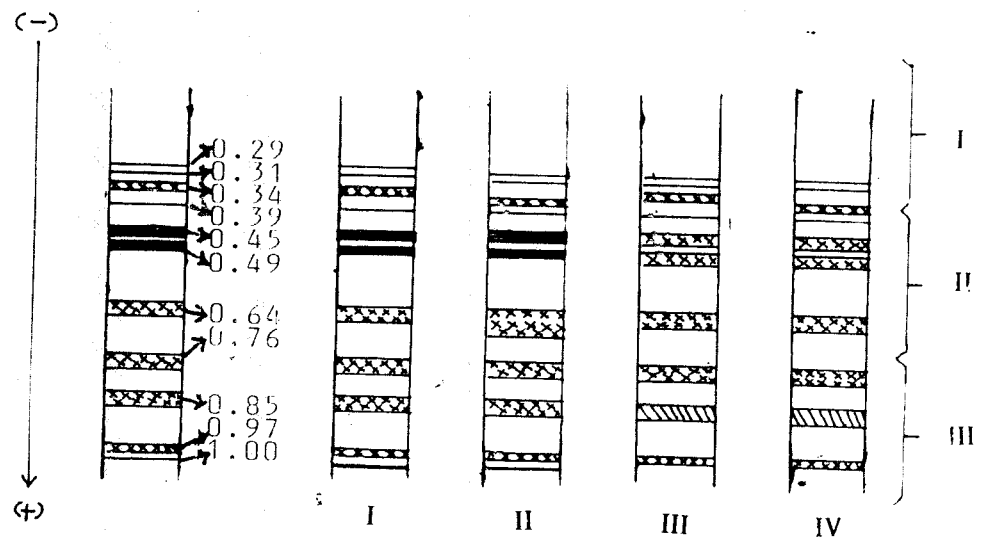


FIG. 21 COMPARATIVE ELECTROPHEROGRAMS OF LIVER PROTEIN PATTERNS IN DIFFERENT SIZE GROUPS OF ETROPLUS MACULATUS

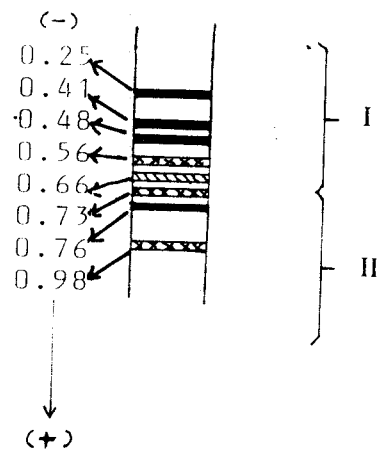


FIG. 22 ELECTROPHEROGRAM OF EYELENS PROTEIN PATTERNS OF ETROPLUS MACULATUS.

were present in stage I, all 4 in stage II & IV and 3 each in stages III and V. Band systems 2 and 5 were the major common bands, with maximum intensities at Rf values of 0.18 and 0.34 respectively at different stages of maturity. Intensities of bands also varied between different stages. Stage I consisted of 6 band systems; Stage II, 8 band systems; Stage III, 9 band systems; Stage IV, 9 band systems and Stage V, 8 band systems (Plate I, Fig. 23).

Comparative protein patterns in different tissues of  
A. maculatus

Protein patterns of muscle, liver, eye lens and ovary showed marked variation in number of bands, their Rf values and staining intensities (Plate X, Fig. 24, Table 48). No common bands were observed in all tissues. Except in eye lens proteins, bands were represented in all the three zones in other tissues. In eye lens, protein bands were observed in Zones I and II only.

Number of bands observed in different tissues and in different zones were as follows:-

Tissue	Zone I	Zone II	Zone III
Muscle	5	3	2
Liver	2	5	4
Eye lens	5	3	-
Ovary	*2	4	3

Includes  
\* 4 component bands of band No.1.

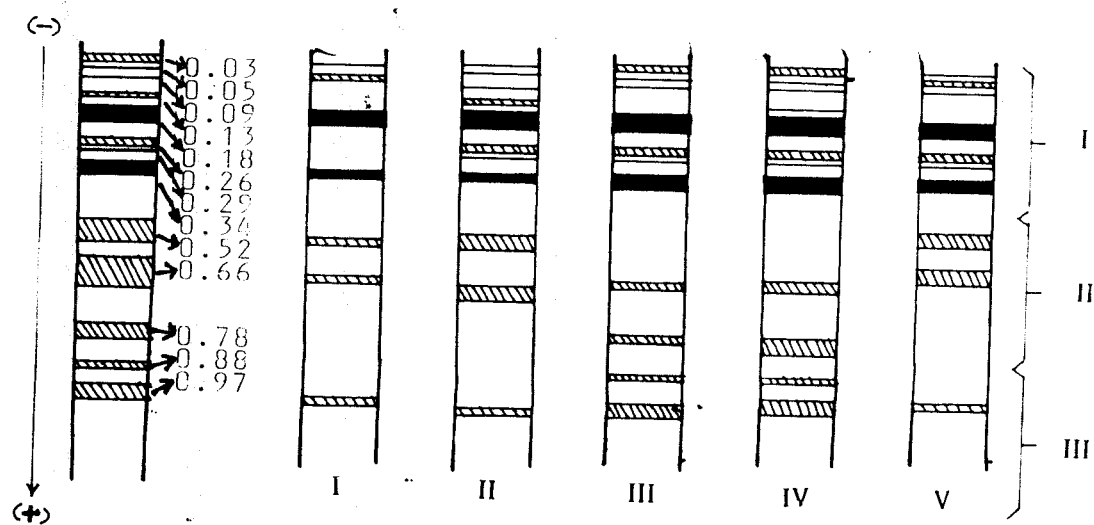


FIG. 23 COMPARATIVE ELECTROPHEROGRAMS OF OVARY PROTEIN PATTERNS AT DIFFERENT STAGES OF MATURITY IN ETROPLUS MACULATUS



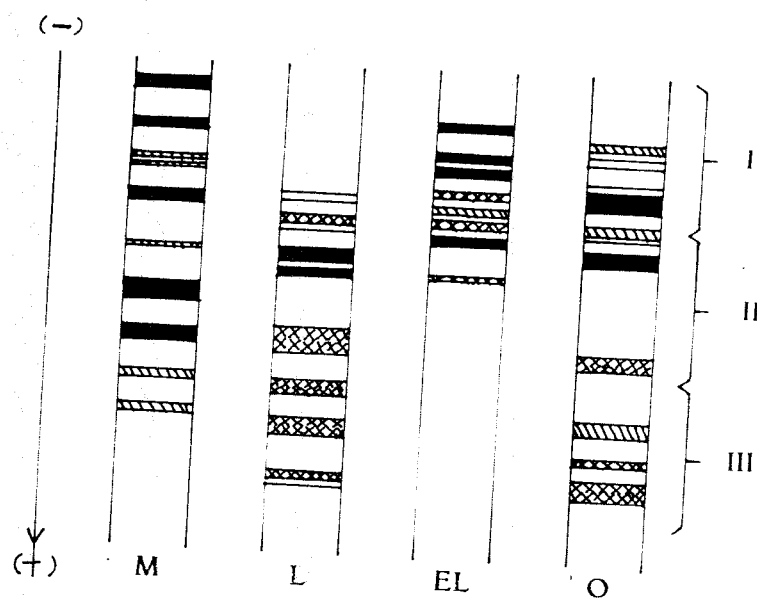


FIG. 24 COMPARATIVE ELECTROPHEROGRAMS OF MUSCLE, LIVER, EYELENS AND OVARY PROTEIN PATTERNS OF ETROPLUS MACULATUS

TABLE - 48 NUMBER OF PROTEIN FRACTIONS, THEIR RELATIVE REACTION VALUES (RF) AND INTENSITIES IN DIFFERENT TISSUES OF L. MACULATUS

[illegible]

### General, Lipo and Glyco protein patterns

A comparison of general, protein, lipo-protein and glyco-proteins of muscle, liver, eye lens and ovary of E. maculatus revealed that the lipo and glyco-proteins had the same mobilities as in general protein fractions (Fig.25). Totally 3 fractions of lipo-proteins were observed in muscle, 4 in liver, 5 in eye lens and 4 in ovary, while 2 fractions of glycoprotein in muscle 3 in liver, 7 in eye lens and 4 in ovary.

In muscle, liver, eye lens and ovary, fractions of glyco and lipo-proteins were observed in I and II zones, only

### Comparison of protein patterns between species

#### Variation between different tissues

Electrophoretic protein patterns of muscle, liver, eye lens and ovary of E. muriei and E. maculatus showed marked variation in the number of protein fractions, their Rf values and intensities of bands (Plate XI, Fig. 26, Table 49). However, Rf values of some of the bands were similar in both species, as can be seen from the following table.

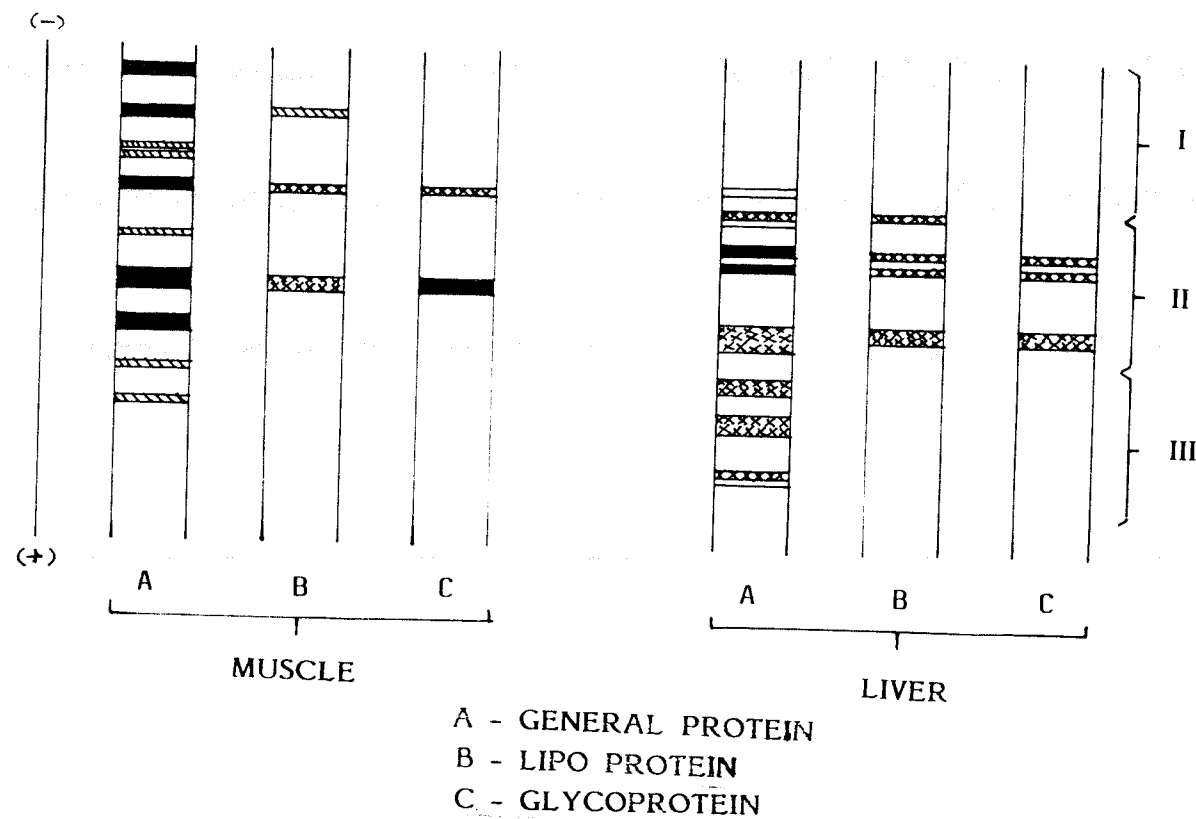


FIG. 25a COMPARATIVE ELECTROPHEROGRAMS OF GENERAL, LIPO AND GLYCO-PROTEIN PATTERNS OF MUSCLE AND LIVER OF ETROPLUS MACULATUS

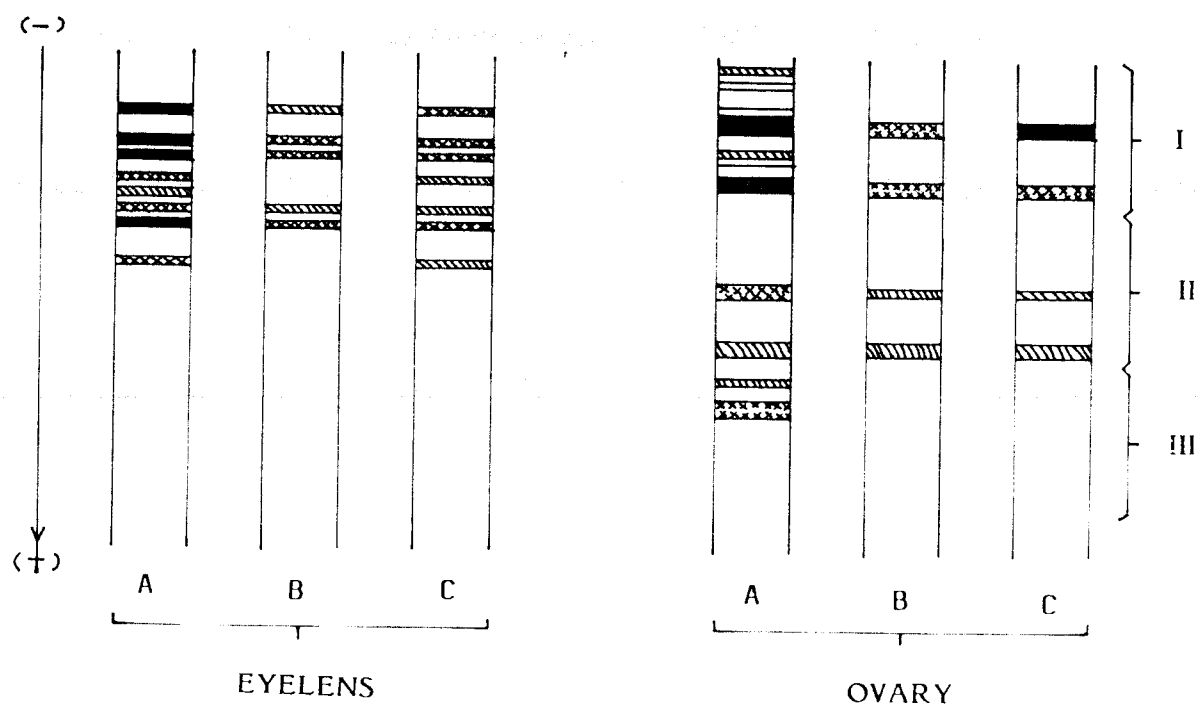


FIG. 25b COMPARATIVE ELECTROPHEROGRAMS OF GENERAL, LIPO AND GLYCO-PROTEIN PATTERNS OF EYELENS AND OVARY OF ETROPLUS MACULATUS.

TABLE - 49 NUMBER OF PROTEIN BANDS, THEIR RELATIVE FRACTION VALUES (Rf) AND INTENSITIES IN DIFFERENT TISSUES OF E. SURATENSIS AND E. MACULATUS

Band No.	MUSCLE				LIVER			
	<u>E. SURATENSIS</u>		<u>E. MACULATUS</u>		<u>E. SURATENSIS</u>		<u>E. MACULATUS</u>	
	Rf	Intensity	Rf	Intensity	Rf	Intensity	Rf	Intensity
1	0.16	XXXXX	0.04	XXXX	0.10	X	0.30	X
2	0.19	XXXX	0.18	XXXX	0.18	X	0.32	X
3	0.25	XXXX	0.28	XX	0.22	XX	0.36	XXXX
4	0.28	XXXX	0.30	XX	0.26	XX	0.39	X
5	0.30	XXXX	0.38	XXXX	0.34	XXXX	0.45	XXXX
6	0.33	XXXX	0.52	XXXX	0.42	XXX	0.49	XXXX
7	0.42	XXX	0.66	XXXX	0.46	X	0.65	XXX
					0.48	X		
8	0.63	XXXX	0.77	XXXX	0.51	XXX	0.76	XXX
9	0.77	XX	0.89	XX	0.59	XX	0.85	XXX
10	0.98	XX	0.98	XX	0.64	XXXX	0.97	XXX
11					0.69	XXX		
12					0.77	XX		
13					0.82	XXX		
14					0.88	XX		
15					0.93	XX		
16					0.99	XX		

SYS LINE					CITY				
Band No.	E. SUBSTRATE		E. FACULATIVE		Band No.	E. SUBSTRATE		E. FACULATIVE	
	Rf	Intensity	Rf	Intensity		Rf	Intensity	Rf	Intensity
2	0.26	XXXX	0.26	XXXX	1 a	0.04	X	0.03	XX
2	0.32	XXXX	0.41	XXXX	b	0.05	X	0.06	X
3	0.42	XX	0.48	XXXX	c	0.07	X	0.08	X
4	0.48	XXXX	0.58	XXX	d	0.09	X	0.13	X
5	0.67	XXXX	0.66	XX	2	0.17	XXXX	0.18	XXXX
6	0.73	XXXX	0.73	XXX	3	0.28	X	0.26	XX
7	0.97	XXX	0.76	XXXX	4	0.33	XXXX	0.29	X
8			0.96	XXX	5	0.40	XX	0.34	XXXX
					6	0.46	XX	0.63	XXX
					7	0.56	XX	0.79	XX
					8	0.58	XX	0.88	XX
					9	0.64	XXXX	0.95	XXX
					10	0.78	XX		
					11	0.87	XX		
					12	0.93	XX		
					13	0.99	XX		

Similarity in Rf values of protein fractions in different tissues of E. suratensis and E. maculatus.

Muscle		Liver		Eye lens		Ovary	
Band	Nos.	Band	Nos.	Band	Nos.	Band	Nos.
E 1	E 2	E 1	E 2	E 1	E 2	E 1	E 2
2	2	12 13	7	1	1	2	2
4	3	14	8	3	3	4	5
5	4	16	9			8	6
8	7	10	10			11	9
9	8						
10	10						

It was also observed that protein fractions of liver tissues of Litropus maculatus migrate to more distance than in E. suratensis. Protein fractions of all tissues were present in all the three zones in E. suratensis and E. maculatus except in eye lens protein fractions, which were present only in Zone I and Zone II (Plate XI and Fig. 26).

#### 6.3.2

#### Protein pattern variation of muscle tissue between different geographical areas

##### Litropus suratensis

Muscle proteins of 8 different geographical areas i.e., I-Cochin, II-Mangalore, III-Marwar, IV-Goa, V-Pondicerry, VI-Muthukadu, VII-Pulikat lake and VIII-Hyderabad were analysed.



A comparison of electrophoretic protein patterns of muscle observed in different geographical areas are presented in plate XII, Fig. 27 and Table 50. It is evident that band Nos. 6, 11 and 12 were present in all areas. Band No. 11 was found to be a major common band with maximum intensity at  $R_f$  values of 0.61-0.62.

Band Nos. 6 and 7 appeared as a single band in area VI (Muthukadu), Band No. 6 appears as single or double bands in samples of Cochin area, indicating its polymorphic nature. Since no genetic system could be evolved from the patterns observed, it is not possible to give genetic interpretation of these variation.

The following bands appeared as double bands in specimens from different areas.

	Area Nos.							
	I	II	III	IV	V	VI	VII	VIII
Band Nos	4,5,6	13	13	12	6	-	-	6

#### *Etroplus maculatus*

Electrophoretic protein patterns of muscle tissues of *E. maculatus* of Cochin, Madras, Pulikat lake and Hyderabad stations revealed considerable variations in the number and staining intensities of bands between different geographical areas. Band Nos. 6, 10 and 11 were common in all areas with band No. 10 as the major common band with maximum intensity

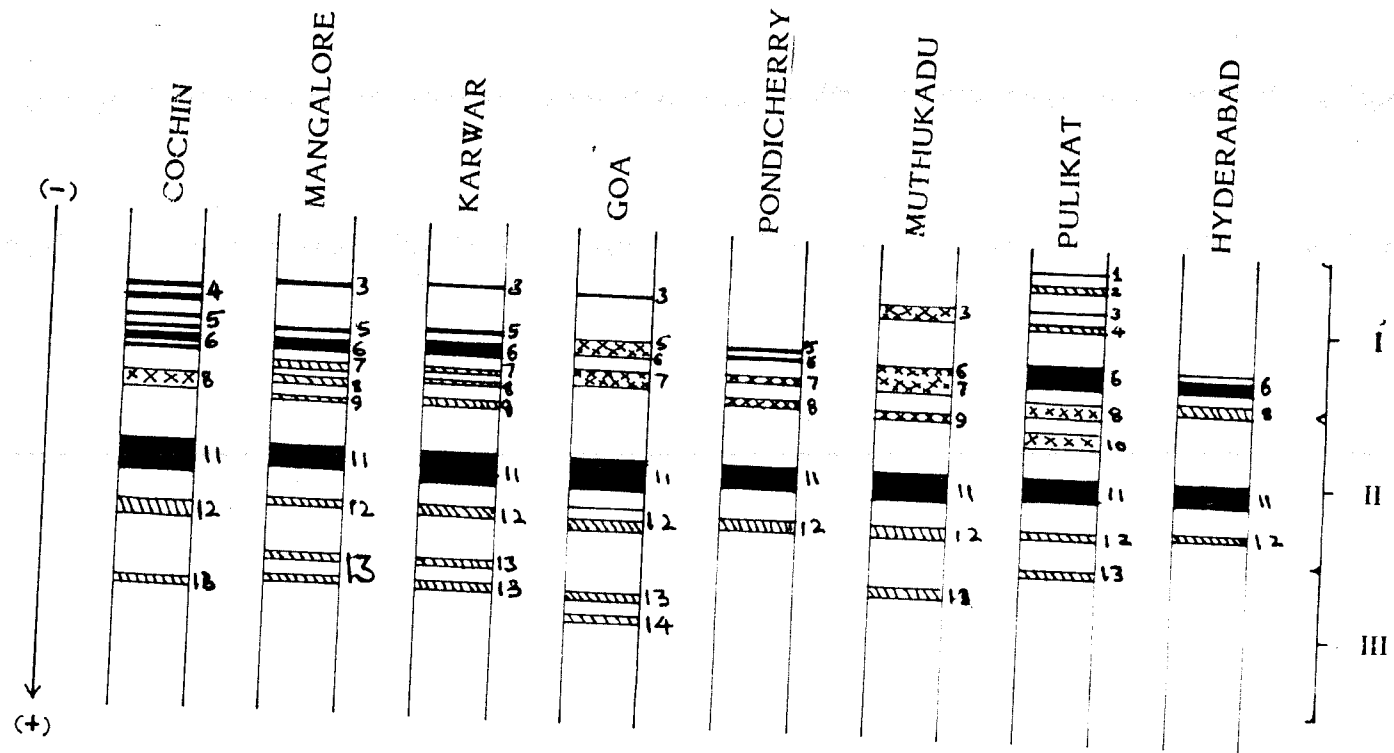


FIG. 27 COMPARATIVE ELECTROPHEROGRAMS OF MUSCLE PROTEINS OF *ETROPLUS SURATENSIS* FROM DIFFERENT LOCALITIES

TABLE - 50 NUMBER OF PROTEIN FRACTIONS OF MUSCLE OF E. SURATENSIS, THEIR Rf  
VALUES AND STAINING INTENSITIES IN DIFFERENT GEOGRAPHICAL AREAS.

Band No.	COCHIN		MANGALORE		KARNAR		GOA	
	Rf	Intensity	Rf	Intensity	Rf	Intensity	Rf	Intensity
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	0.16	XXXX	0.16	XXXX	0.16	XXXX
4 a	0.18	XXXX	-	-	-	-	-	-
b	0.21	XXXX	-	-	-	-	-	-
5 a	0.26	XXXX	-	-	-	-	-	-
b	0.28	XXXX	0.28	XXXX	0.28	XXXX	-	-
6 a	0.31	XXXX	-	-	-	-	-	-
b	0.34	XXXX	0.32	XXXX	0.31	XXXX	0.30	XXX
7	-	-	0.37	XXX	0.36	XX	0.37	XXX
8	0.42	XXX	0.41	XX	0.40	XX	-	-
9	-	-	0.46	XX	0.45	XX	-	-
10	0.61	XXXX	0.61	XXXX	0.62	XXXX	0.62	XXXX
11	0.74	XX	0.72	XX	0.73	XX	0.70 0.75	II XX
13 a	-	-	0.86	XX	0.86	XX	-	-
b	-	-	0.92	XX	0.92	XX	-	-
14	0.94	XX	-	-	-	-	0.93 0.99	XX XX
15	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-

Band No	Pondicherry		Muthukadu		Pulikat lake		Hyderabad	
	Rf	Intensity	Rf	Intensity	Rf	Intensity	Rf	Intensity
1	-	-	-	-	0.029 0.058	x x	-	-
2	-	-	-	-	0.09	xx	-	-
3	-	-	0.17	xxx	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	0.28	xxxx	-	-	-	-	0.30	xxxx
7	0.30	xxxx	0.34	xxx	0.32	xxxxx	0.33	xxxxx
7	0.35	xxx	0.34	xxx	-	-	-	-
8	0.41	xxx	0.43	xxx	0.40	xxx	0.39	xxx
9	-	-	-	-	0.48	xxx	-	-
10	0.61	xxxxx	0.61	xxxxx	0.61	xxxxx	0.61	xxxxx
11	0.71	xx	0.75	xxx	0.72	xx	0.71	xx
12	-	-	-	-	0.82	xx	-	-
13	-	-	0.88	xx	-	-	-	-
14								
15								
16								
17								

TABLE - 51 NUMBER OF MUSCLE PROTEIN FRAGMENTS OF *LODUS* sp. INTENSITIES OF B. MACULATUS IN DIFFERENT GEOGRAPHICAL AREAS.

Band No.	Cochin		Mathukadu		Pulikat lake		Hyderabad	
	Rf	Intensity	Rf	Intensity	Rf	Intensity	Rf	Intensity
1	0.05	XXXX	-	-	-	-	-	-
2	-	-	0.11	XXX	0.10	XXX	-	-
3	0.19	XXXXX	-	-	-	-	-	-
4	-	-	0.23	XXX	0.24	XXX	-	-
5 a	0.29	XX	-	-	-	-	0.27	XXXXX
b	0.31	XX	-	-	-	-	0.32	XXXXX
6 a	0.41	XXXXX	0.35	XXXXX	0.35	XXXXX	0.40	XXX
b	-	-	0.41	XXXXX	-	-	-	-
7	-	-	0.47	XX	-	-	0.45	XXXXX
8	0.54	XXXXX	-	-	0.52	XXXXX	-	-
9	-	-	0.56	XXXXX	-	-	-	-
10	0.65	XXXXX	0.66	XXXXX	0.66	XXXXX	0.66	XXXXX
11	0.78	XXXXX	0.77	XXXXX	0.77	XXX	0.77	XXX
12	0.90	XX	0.88	XX	0.88	XX	-	-
13	0.98	XX	0.96	XX	0.98	XX	-	-

at  $R_f$  value of 0.65 to 0.66 (Plate XIII, Fig. 28, Table 51).

### 6.3.3. Enzyme expression in tissues

#### Expression of Alcohol dehydrogenase (Adh)

##### *Ectophasia surinensis*

A comparison of Adh resolved in Tris-glycine-HCl buffer is presented in Plate XIV and Fig. 29. Twelve different tissues were analysed to see the expression of Adh. It is seen that the patterns of Adh consisted of 11 bands. No common bands could be observed. Adh activity was found to be more in liver tissue than in other tissues. Band No. 4 in liver tissue appeared as the darkest band. Since no uniform band patterns could be observed due to difference in the number of bands between tissues, the number of loci present could not be detected.

##### *Ectophasia maculatus*

Adh patterns in different tissues of *E. maculatus* as resolved in Tris-HCl-Glycine buffer showed 8 banding patterns, (Plate V, Fig. 30). Of these band No.2 predominated in most of the tissues, Band No.6 appeared as the darkest band in liver tissue, while band No.2 and 4 in ovary tissue. Due to difference in the number of bands between tissues no uniform band patterns could be observed, hence the number of loci present could not be detected.

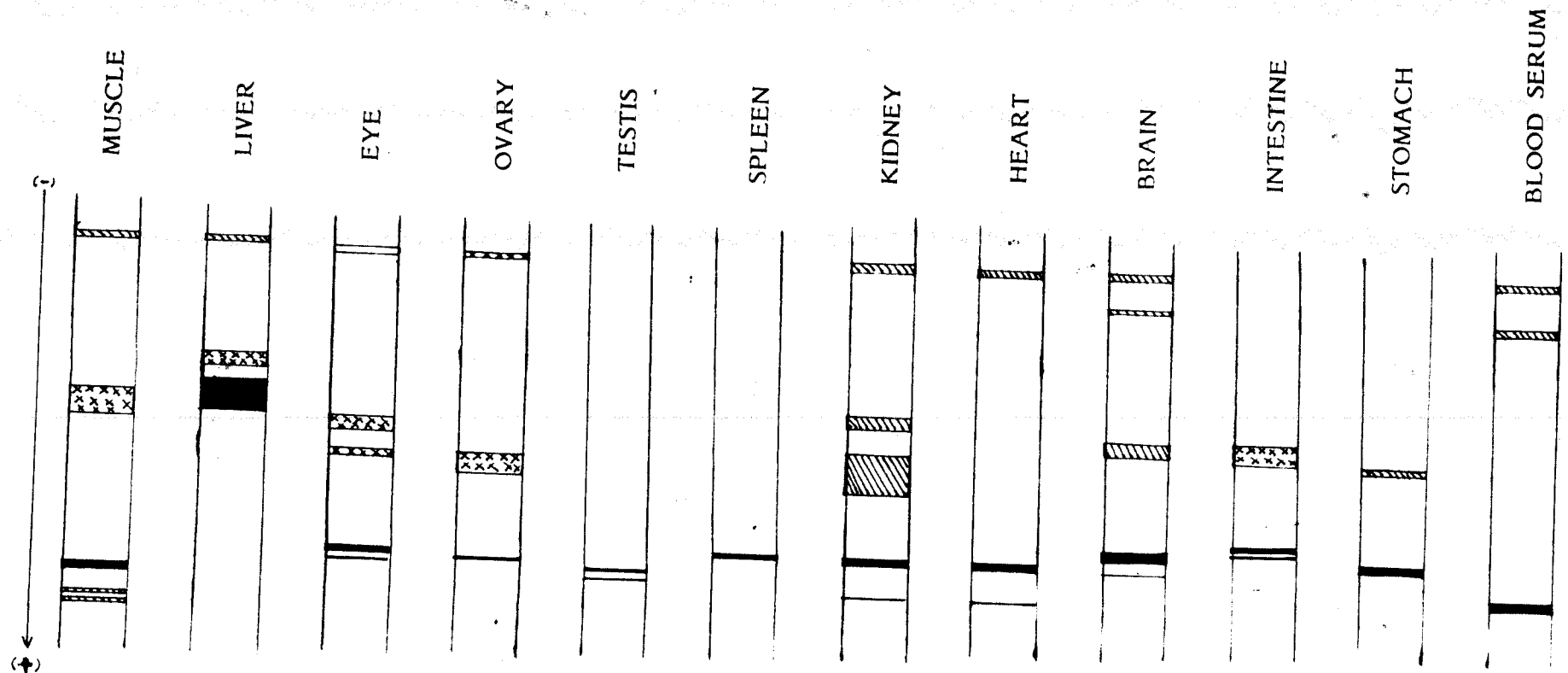


FIG. 29 EXPRESSION OF ALCOHOL DEHYDROGENASE IN DIFFERENT TISSUES OF ETROPLUS SURATENSIS

### Expression of Malate Dehydrogenase (Mdh)

#### Etropius suratensis

Studies on Mdh patterns in different tissues of E. suratensis revealed a total of 9 bands (Plate XVI, Fig.31) Band Nos. 4 and 6 were common in most of the tissues. In muscle tissue patterns, band Nos. 4 and 7 appeared as darkest bands. Band No. 4 predominated in muscle, liver, eye, ovary, testis, spleen, kidney, heart, brain, intestine and stomach. Band No. 5 was absent in muscle and spleen. Due to difference in the number of bands between tissues, uniform band pattern could not be established. Thereafter, number of loci could not be identified.

#### Etropius maculatus

Nine tissues were analysed to determine the expression of Mdh. It was observed (Plate XVII Fig. 32) that the band No. 9 was present in all tissues except in liver. Eye, ovary, spleen and kidney consisted of maximum number of bands. No uniform patterns could be observed due to difference in the number of bands between tissues, making it difficult to identify the number of loci present.

### Expression of malic enzyme (Me)

#### Etropius suratensis

A comparison of Me patterns observed in different tissues of E. suratensis is presented in Plate XVIII and Fig. 33.



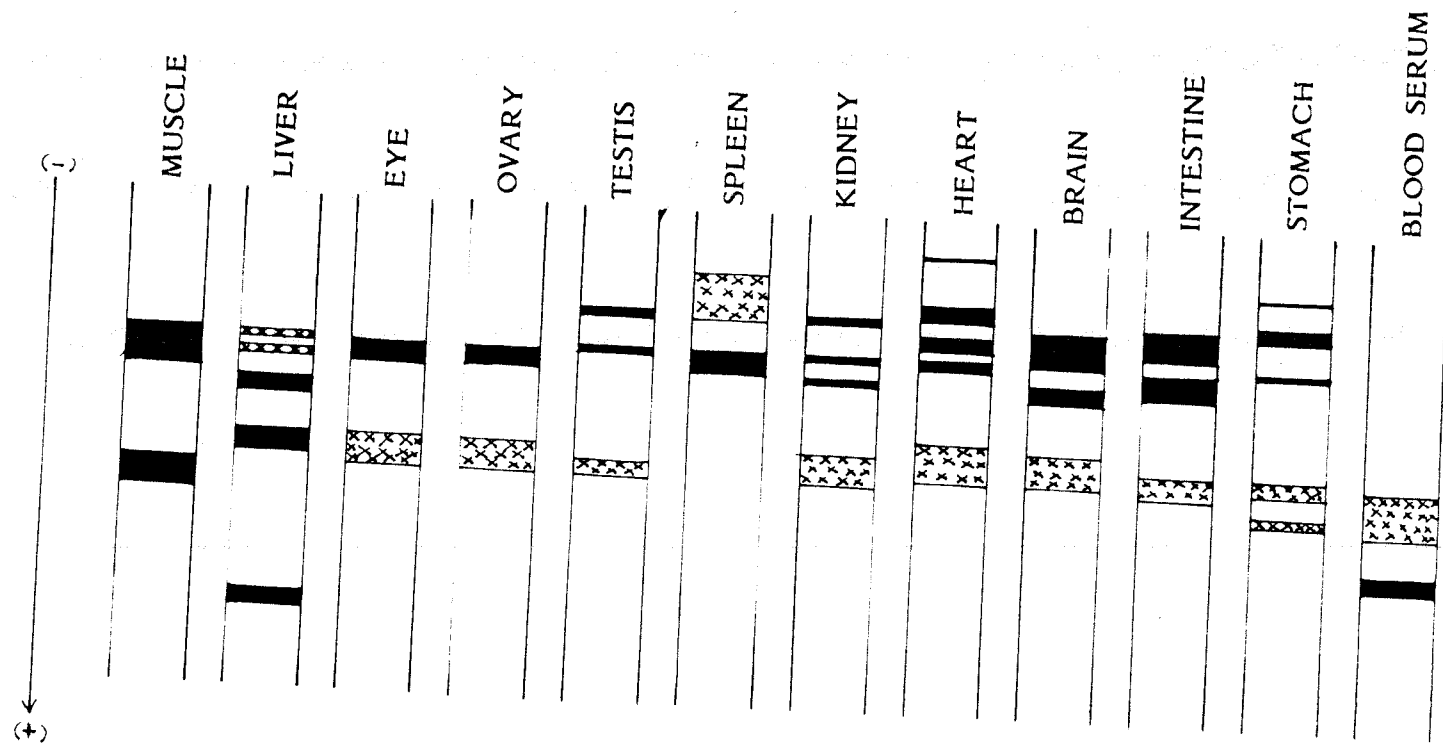


FIG. 31 EXPRESSION OF MALATE DEHYDROGENASE IN DIFFERENT TISSUES OF ETROPLUS SURATENSIS

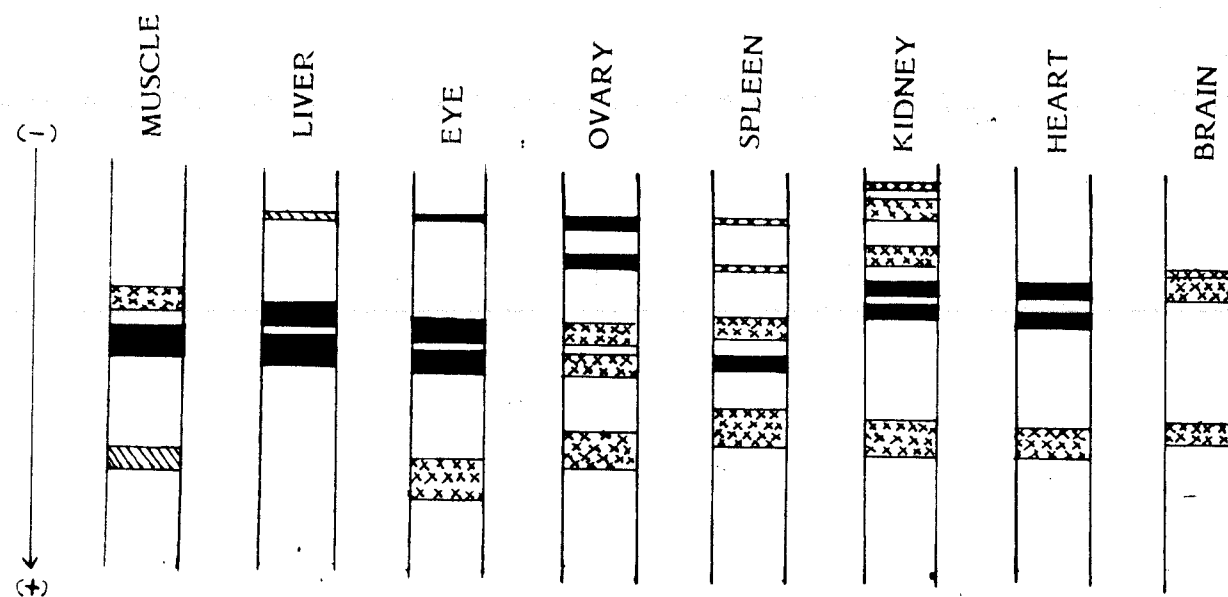


FIG. 32 EXPRESSION OF MALATE DEHYDROGENASE IN DIFFERENT TISSUES OF  
ETROPLUS MACULATUS

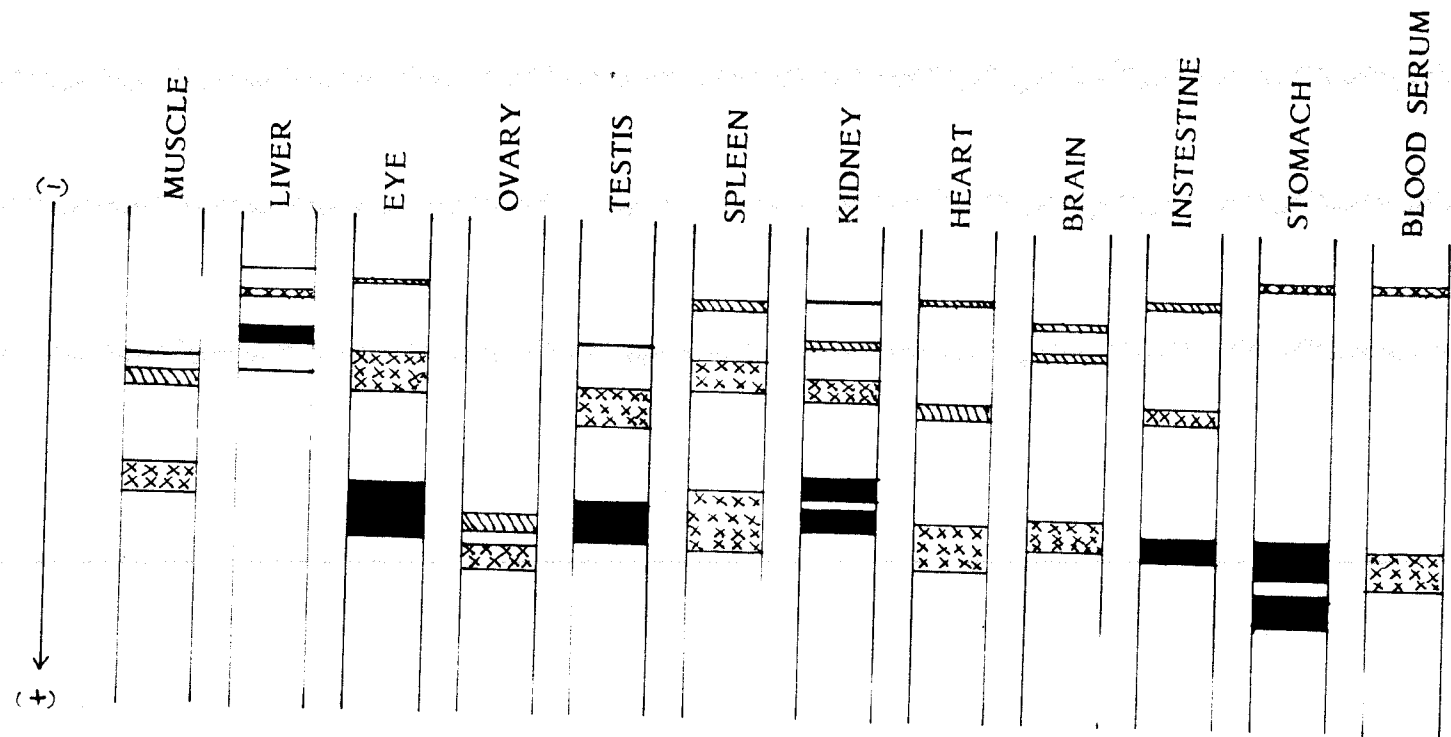


FIG. 33 EXPRESSION OF MALIC ENZYME IN DIFFERENT TISSUES OF ETROPLUS SURATENSIS

A total of 10 bands were observed in 12 different tissues. Bands Nos. 2, 5 and 8 were common in maximum tissues. Due to difference in the number of bands between tissues, uniform band pattern could not be established so the number of loci present could not be detected.

### Etropolis maculatus

The patterns in different tissues of E. maculatus showed 12 bands (Plate XIX, Fig. 34). It is seen that band Nos. 1, 5 and 10 were common in most of the tissues. Band No. 3 in liver and Band No. 1 and 3 in ovary were the darkest bands. No loci could be detected because of the difference observed in the number of bands between the tissues.

### Expression of Lactate Dehydrogenase (Ldh)

Different tissues of E. suratensis and E. maculatus were analysed to detect the expression of Ldh. A comparison of Ldh patterns is presented in Plate XX and Figs. 35, 36.

Five tetrameric Ldh bands were observed in eye tissues of both species. Each species had 5 distinct bands. The slower band was identified as the homotetrameric expression of Ldh-1 or A locus; the fastest one as the homotetrameric expression of Ldh-2 or B locus and the intermediate three bands, as the heterotetramers of locus A and B. All five bands were termed as  $A_4$ ,  $A_3B_1$ ,  $A_2B_2$ ,  $A_1B_3$  and  $B_4$ . The nomenclature of the bands is based on Bailey and Wilson (1968).

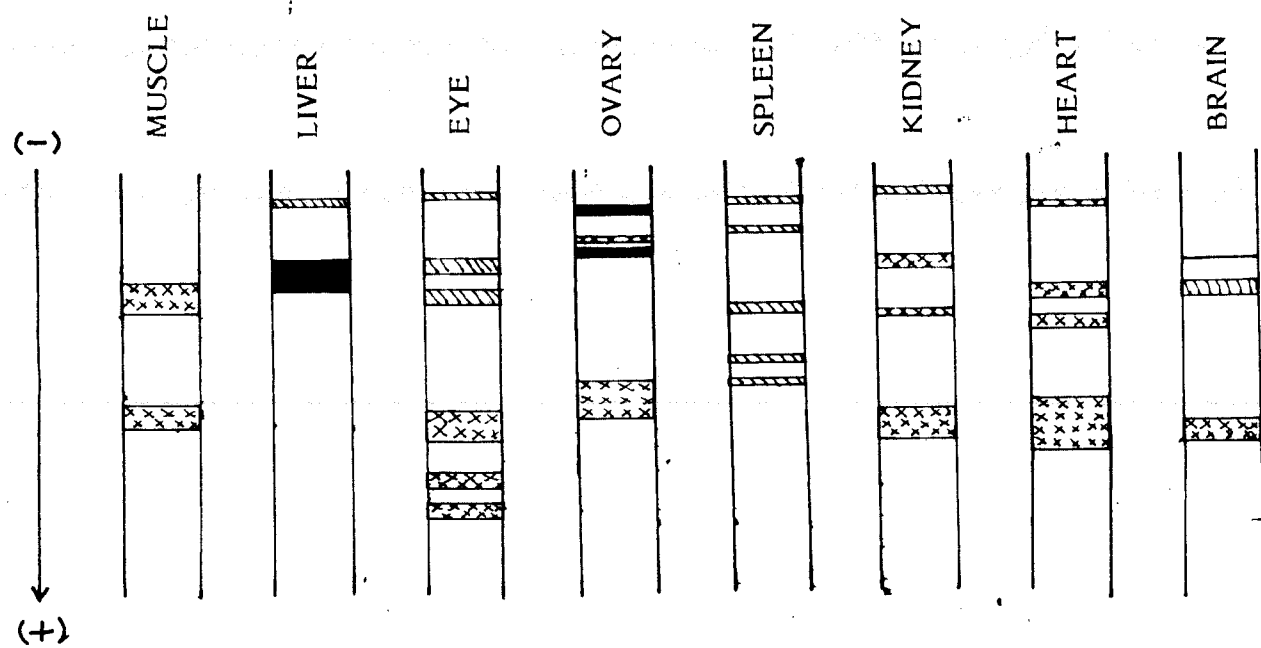


FIG. 34 EXPRESSION OF MALIC ENZYME IN DIFFERENT TISSUES OF ETROPLUS  
MACULATUS

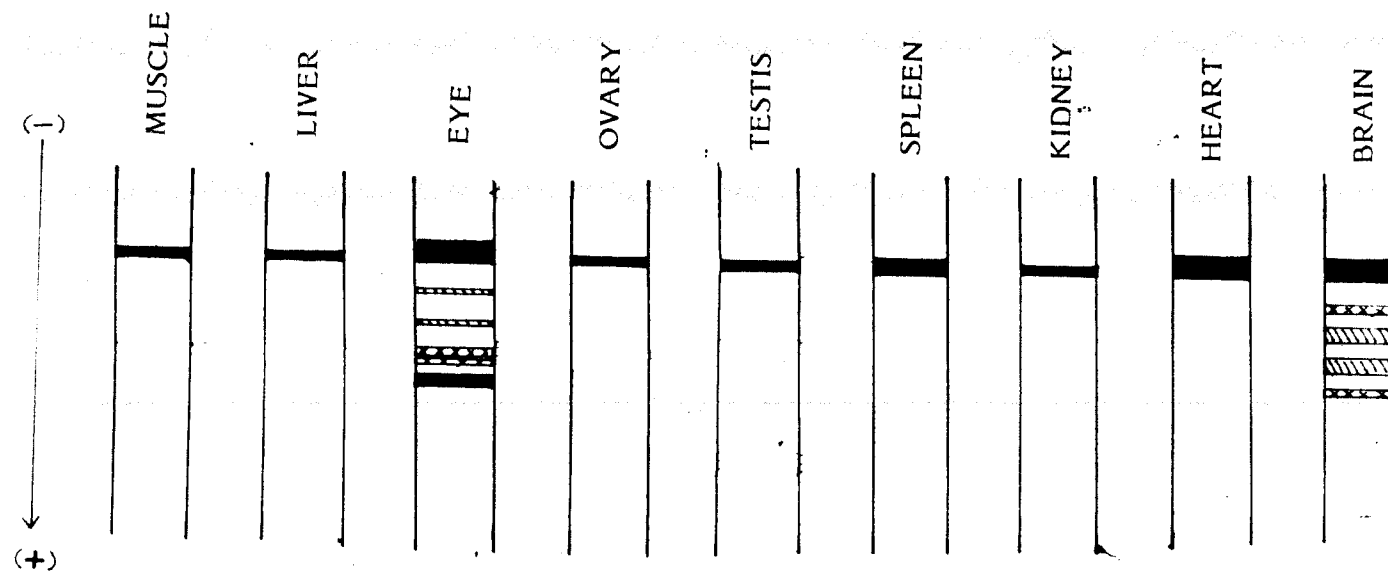


FIG. 36 EXPRESSION OF LACTATE DEHYDROGENASE IN DIFFERENT TISSUES OF ETROPLUS MACULATUS

Locus A predominated in all tissues examined but locus B was present in only eye tissues in both species. Heterotetramers of locus A were observed in brain tissue of both species.

Ldn patterns varied in their Rf values in E. suratensis and E. maculatus. No variation could be observed in juveniles and adults of both species.

#### Expression of Esterase (Est.)

##### Etroplus suratensis

The details of Est patterns observed in different tissues of E. suratensis are presented in Plate XXI and Fig. 37. It was observed that band No. 10 was common in all tissues and appeared as dark band. A total of 14 band systems were observed with maximum 7 bands in liver tissue.

##### Etroplus maculatus

A comparison of Est patterns in different tissues of E. maculatus is presented in Plate XXII and Fig. 38. Fourteen band systems were observed. A maximum of 7 band systems were observed in liver tissue. Band system 11 was present in all tissues.

#### Expression of acid phosphatase (ACP)

##### Etroplus suratensis

Acid phosphatase was resolved in Tris-maleic-EDTA buffer

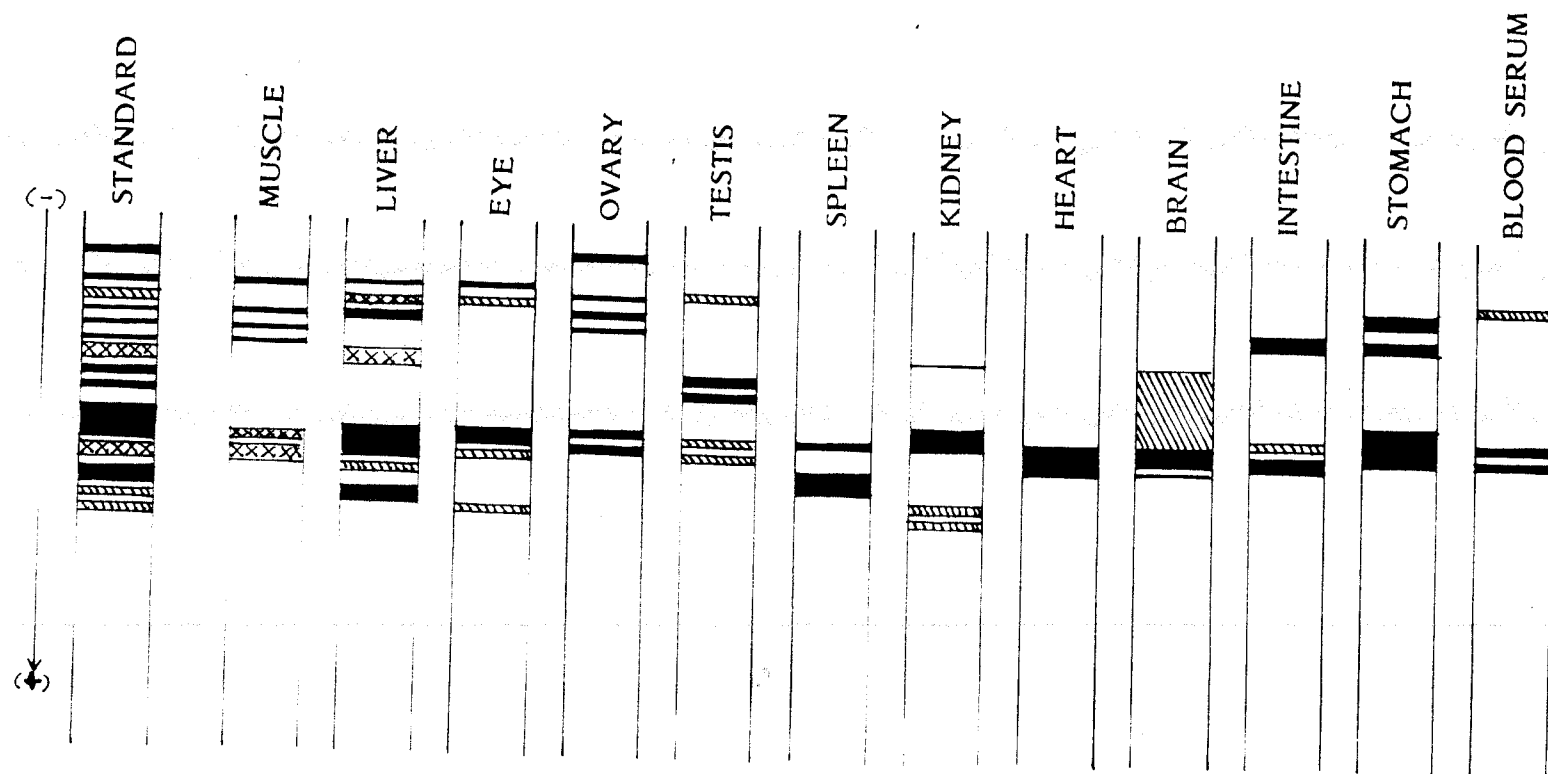


FIG. 37 EXPRESSION OF ESTERASE IN DIFFERENT TISSUES OF ETROPLUS SURATENSIS



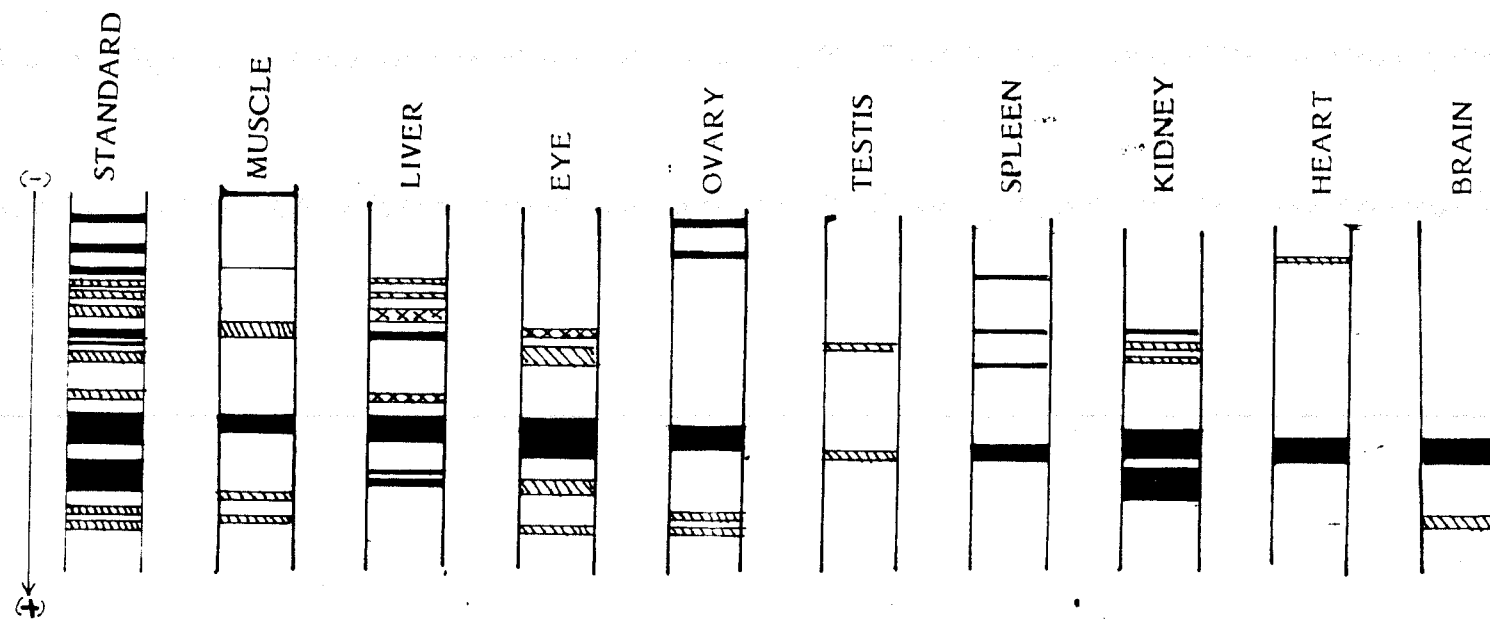


FIG. 38 EXPRESSION OF ESTERASE IN DIFFERENT TISSUES OF ETROPLUS MACULATUS

and its expression on different tissues is presented in Plate XXIII and Fig. 39. It was observed that two bands occur in all tissues of E. suratensis. Band No. 1 was the darkest band in all tissues and band No. 2 was lightly stained. In liver tissue both bands were darkly stained.

#### Etroplus maculatus

A comparison of Acph in different tissues of E. maculatus is presented in Plate XXIV and Fig. 40. It was observed that all tissues consisted of two bands. Electrophoretic mobilities and intensities of these bands differ between tissues; liver tissue exhibiting darkest bands.

Juveniles and adults of both E. suratensis and E. maculatus were tested to see the variation in Ldh, Acph and Est patterns. Eye tissues were used for Ldh, and liver for Acph and Est. No difference could be detected in both species in isoenzyme patterns in these stages.

#### 6.3.4. Genetic variation

##### Lactate dehydrogenase

##### Etroplus suratensis

Overall incidence of heterozygosity was determined at Ldh-1 locus in 145 specimens collected from Cochin backwaters. Study revealed two loci in Ldh system in eye of E. suratensis. The slow migrating locus was termed as Ldh-1 and fast migrating locus as Ldh-2. It was also observed that Ldh-1 locus is

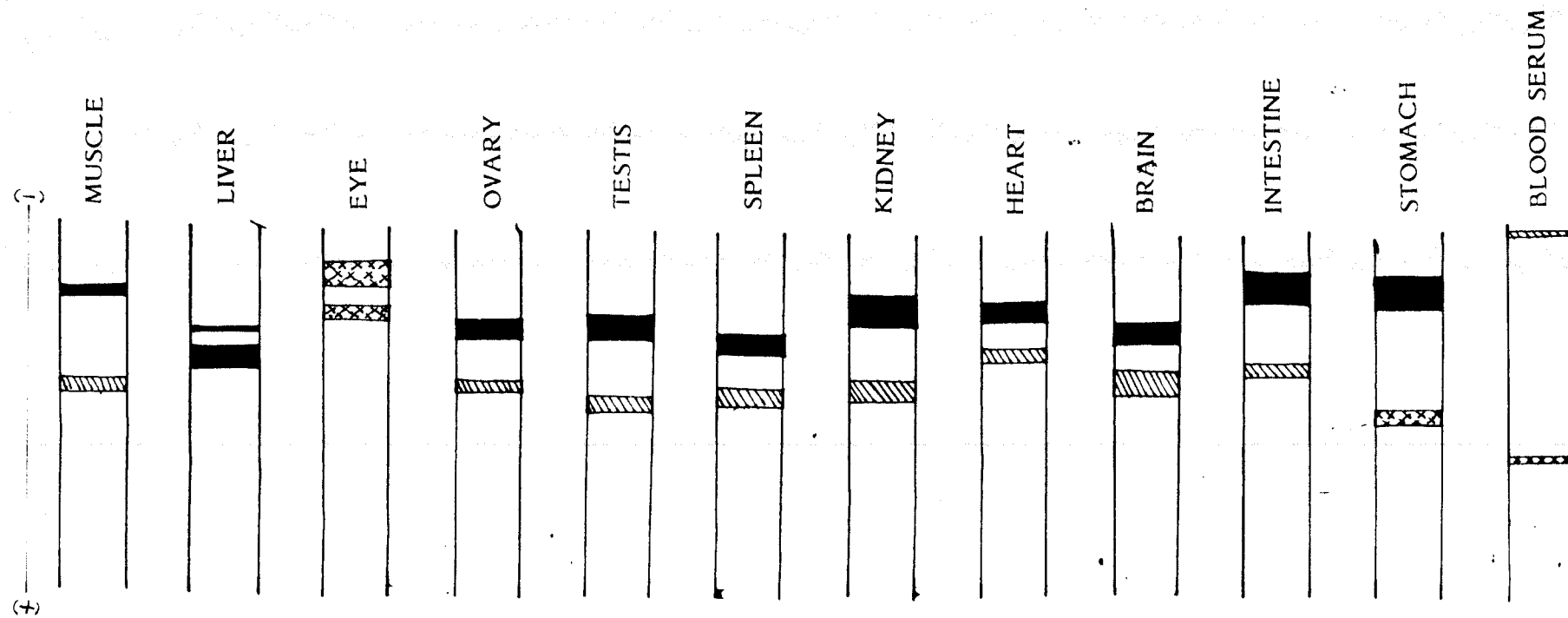


FIG. 39 EXPRESSION OF ACID PHOSPHATASE IN DIFFERENT TISSUES OF ETROPLUS SURATENSIS

polymorphic, while Ldh-2 locus is monomorphic. In the polymorphic loci three alleles were observed.

Ldh patterns of homozygote specimens consisted of two Ldh loci which were presumed to be the homotetramers, whereas the heterotetramers were observed at positions intermediate between these two loci. However, in Ldh patterns of some specimens additional band appeared in between the cathode and Ldh-1 locus. This band was found to occur at two electrophoretic mobilities.

These bands were named as Ldh-1 (95) and Ldh-1 (92) which were considered to be the heterozygotes of Ldh-1 (100). (Plate XXV, Fig. 41).

The distribution of different phenotypes observed in the samples examined was as follows:-

	<u>No. of observations</u>
Ldh-4 (100/100)	68
Ldh-1 (100/ 95)	36
Ldh-1 (100/ 92)	41
Total No. of individuals	145

A comparison of frequency of allele and genotypes observed and expected values of genotypes and chi-square value calculated according to the method described in the section materials and methods, are presented in Table 52.

It is evident from the Table that there is considerable variation between the observed and expected values and these

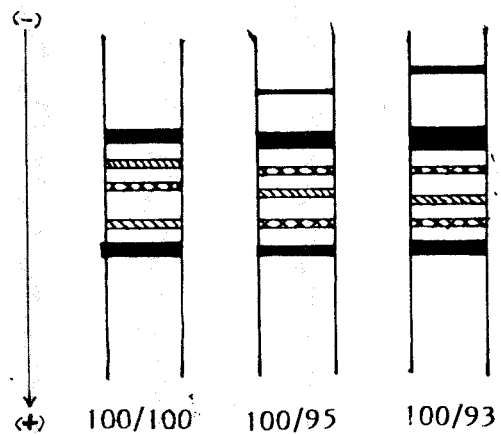


FIG. 41 DIAGRAMATIC REPRESENTATION OF LACTATE DEHYDROGENASE PHENOTYPES IN EYE OF ETROPLUS SURATENSIS

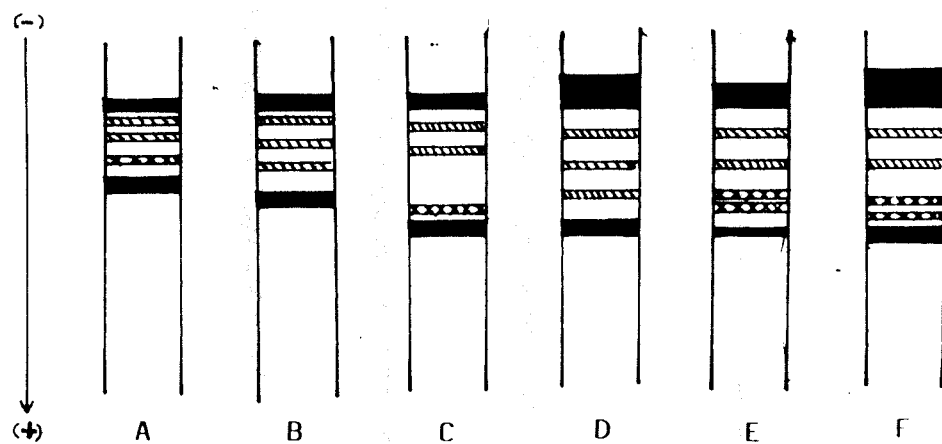


FIG. 42 DIAGRAMATIC REPRESENTATION OF LACTATE DEHYDROGENASE PHENOTYPES IN EYE OF ETROPLUS MACULATUS

TABLE - 52 COMPARISON OF OBSERVED ZYGOTIC FREQUENCIES AND THEIR HARDY WEINBERG EXPECTATIONS AT THE LDH-A LOCUS FOR SAMPLES OF E. SURATENSIS.

Alleles	Frequency of allele	Genotype	Frequency of genotype	Values of genotypes		χ <sup>2</sup>
				Observed	Expected	
100	0.7344	100/100	0.5393	68	78.19	1.33
		100/95	0.1822	36	26.41	3.47
95	0.1261	95/95	0.0154	00	02.23	2.23
		100/92	0.2075	41	30.08	3.95
92	0.1413	95/92	0.3050	00	05.07	5.07
		92/92	0.0199	00	02.89	2.89
Total					10.94	
P					0.01	

Observed heterozygosity  $H_o = 0.5310$

Expected heterozygosity  $H_e = 0.4245$

D = +0.250

differences are highly significant ( $P < 0.1$ ). An excess of heterozygosity ( $D = +0.250$ ) was also observed.

### Etroplus maculatus

Ldh patterns of E. maculatus and E. suratensis were virtually identical. Ldh pattern consisted of five bands, two dark bands, slower and faster, being the homotetramers, and three light bands in the position intermediate to the dark bands being the heterotetramers. In some specimens 4 heterotetramers with a total of 6 bands were observed (Fig. 42, Table 53). No additional bands were observed which could have given the evidence of heterozygote, however, the mobilities of bands varied in a number of samples. Since no heterozygote patterns could be observed, it is not possible to give a genetic interpretation for genetic variation at Ldh locus in this species.

### Esterase

#### Etroplus suratensis

Esterases are complex isoenzymes and consists of multi-locus systems. In the present study esterase bands obtained in different tissues were grouped into 14 banding systems. Maximum bands were obtained in liver, which produced complex structure. Band system 13 was found to be polymorphic in this species (Fig. 43). Liver was tested in 168 specimens and three different types of phenotypes controlled by two alleles A and B were observed. Allelic and genotype

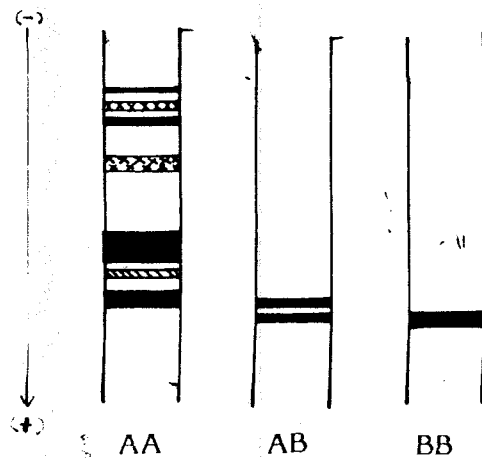


FIG. 43 DIAGRAMATIC REPRESENTATION OF ESTERASE PHENOTYPES  
IN LIVER TISSUE OF ETROPLUS SURATENSIS

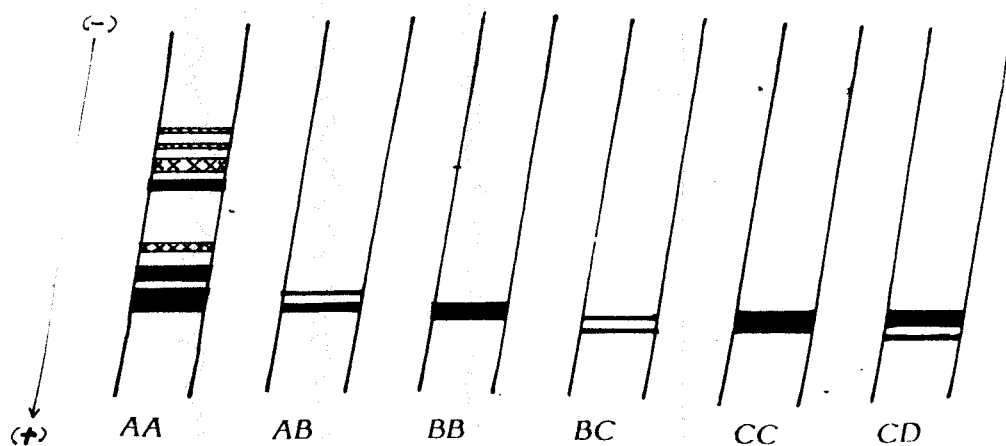


FIG. 44 DIAGRAMATIC REPRESENTATION OF ESTERASE PHENOTYPES  
IN LIVER TISSUE OF ETROPLUS MACULATUS



TABLE - 53 NUMBER AND PERCENTAGE OF Ldh PATTERNS OBSERVED IN EYE  
TISSUE OF E. MACULATUS

Type of patterns	No. of Observations	Percentage
A	12	06.55%
B	89	48.63%
C	35	19.12%
D	09	04.91%
E	21	11.47%
F	17	09.28%

frequencies, observed and expected values of genotype distribution and chi-square values are presented in the Table 54.

It was observed that there is considerable variation between the observed and expected values and these differences were highly significant ( $P < 0.01$ ). An excess in heterozygosity was also observed.

#### Etroplus maculatus

Liver tissue was analysed to score the frequency of different genotypes from 177 specimens. Bands were grouped into 12 systems and the band No. 12 was found to be polymorphic. This band was controlled by 4 alleles giving rise to ten phenotypes, of which six phenotypes have been detected from 177 specimens (Fig. 44). The distribution of 6 phenotypes in the samples analysed was as follows:

<u>Phenotypes</u>	<u>No. of individuals</u>
95/95	12
95/100	34
100/100	63
100/110	29
110/110	21
110/120	18
	<hr/>
	<b>n= 177</b>
	<hr/>

TABLE - 54 COMPARISON OF OBSERVED ZYGOTIC FREQUENCIES AND THEIR HARDY WEINBERG EXPECTATIONS AT THE ESTERASE LOCUS IN E. SURATENSIS

Allele	Frequency of allele	Genotype	Frequency of genotype	Values of genotypes		$\chi^2$
				Observed	Expected	
100	0.5206	100/100	0.2712	35	45.5616	2.4482
		100/105	0.4989	105	83.8192	6.3545
105	0.4790	105/105	0.2294	28	38.5292	2.8821
					Total	10.6848
					$P < 0.01$	

Observed heterozygosity  $H_o = 0.6250$

Expected heterozygosity  $H_e = 0.4989$

$D = +0.2527$

Allelic frequencies, genotypic frequencies, observed and expected values and chi-square values calculated are presented in Table 55.

It is seen that there is considerable variation between the observed and expected values and these differences were highly significant ( $P < 0.01$ ). A deficit in heterozygosity was also observed.

#### Acid phosphatase

##### *Etrophus suratensis*

Acid phosphatase patterns observed in 152 specimens of liver of *E. suratensis* is presented in plate XVI A Fig. 45 and Table 56. Based on the staining intensities and densities of bands in heterozygote phenotypes, no genetic model could be evolved, therefore it is not possible to give the genetic interpretation of variation for Acph locus in this species.

##### *Etrophus maculatus*

Liver tissues from 178 specimens were analysed to see the occurrence of different phenotypes. Three phenotypes were identified as Acph 100/100, Acph 100/105 and Acph 105/105 (Plate XVI B, Fig. 46; Table-57). Distribution of the phenotypes observed in specimens analysed was as follows:

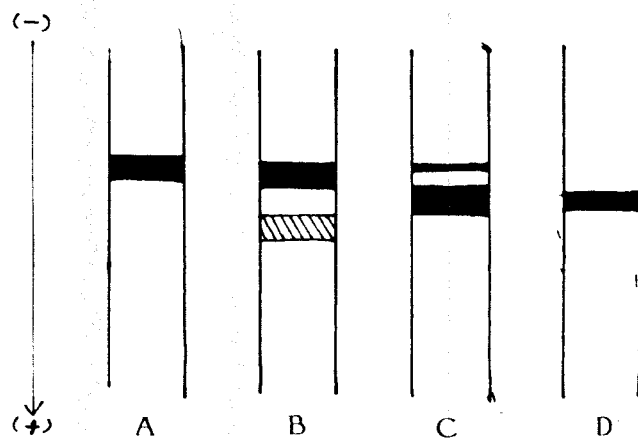


FIG. 45 DIAGRAMATIC REPRESENTATION OF ACID PHOSPHATASE PHENOTYPES IN LIVER TISSUE OF ETROPLUS SURATENSIS

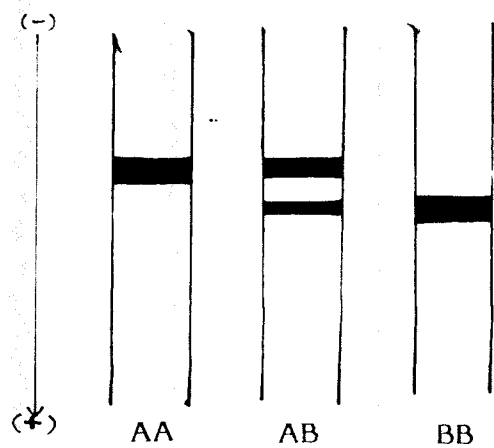


FIG. 46 DIAGRAMATIC REPRESENTATION OF ACID PHOSPHATASE PHENOTYPES IN LIVER TISSUE OF ETROPLUS MACULATUS

TABLE - 55 COMPARISON OF OBSERVED ZYGOTIC FREQUENCIES AND THEIR HARDY WEINBERG EXPECTATIONS AT THE ESTERASE LOCUS IN *E. MACULATUS*.

Allele	Frequency of allele	Genotype	Frequency of genotype	Value of genotype		$\chi^2$
				Observed	Expected	
95	0.1638	95/95	0.0268	12	4.74	11.1002
		95/100	0.1748	34	30.93	00.3047
95	0.5339	95/110	0.0823	00	14.56	14.56
		95/120	0.0166	00	2.93	02.93
100	0.5318	100/100	0.2849	63	50.42	03.13
		100/110	0.2683	29	47.48	07.19
		100/120	0.0542	00	09.59	09.59
110	0.2514	110/110	0.0632	21	11.18	08.60
		110/120	0.0255	18	04.51	40.35
120	0.0508	120/120	0.0025	00	00.44	00.44

<b>Total</b>	<b>98.19</b>
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 $P \leq 0.01$ 

Observed heterozygosity  $H_o = 0.4576$

Expected heterozygosity  $H_e = 0.6214$

D - 0.263

TABLE - 56 NUMBER AND PERCENTAGE OF ACFT PATTERNS OBSERVED IN  
EYE TISSUE OF L. SUBAQUATIC

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Types of patterns	No. of observations	Percentage
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A	29	19.07
B	48	31.57
C	42	27.63
D	33	21.71

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TABLE - 57 COMPARISON OF OBSERVED ZYGOTIC FREQUENCIES AND THEIR HARDY  
WEINBERG EXPECTATIONS AT THE ACIN LOCUS FOR SAMPLES OF E. MACULATUS

Allele	Frequency of allele	Genotype	Frequency of genotype	Value of genotype		$\chi^2$
				Observed	Expected	
100	0.6343	100/100	0.4029	73	71.7162	0.0229
		100/105	0.4635	80	82.503	0.0759
105	0.3651	105/105	0.1332	25	23.7096	0.0706
					Total	0.1694

Observed heterozygosity  $H_o = 0.4494$

Expected heterozygosity  $H_e = 0.4635$



100/100	73
100/105	80
105/105	25
	<hr/>
n =	178

It is evident from the table that there is no difference between observed and expected values of phenotypes, and a deficit in heterozygosity was found, which is not significant.

#### 6.4. Discussion

A single standard method cannot be made use of uniformly for all species, because of species specific effects with respect to buffers, tissues and staining procedures. So, for each species multiple electrophoretic procedures become essential using various combinations of the buffers, gels and staining procedures. In the present study on E. suratensis and E. maculatus modified standard procedure in electrophoresis suitable for the species using polyacrylamide gel technique has been developed.

Whereas, different solvents like sucrose (0.25 M) solution and double distilled water were used by some workers (Bouck and Ball, 1969; Koehn et al., 1971; Ridgway et al., 1970; Utter et al., 1979 and McAndrew and Masumdar, 1983), buffers like phosphate (pH 7.0) and Tris-HCl (pH 7.5) have been used by others (Hodgins et al., 1969; Willisroft and Tsuyuki, 1970; Holmes and Whitt, 1970; Vorwyll, 1979).

For the present study, double distilled water, sucrose 0.25 M, Tris-HCl and phosphate buffers have been tested and double distilled water was found to be suitable for both protein as well as enzymes. Loss of protein bands in buffers and sucrose was presumed due to the denaturation of proteins. Since best resolution was obtained by using double distilled water to extract proteins and enzymes it was used in all experiments.

Since the total protein content in the different tissues vary, different concentrations of the samples were tried. It was observed that in muscle, ovary, eye lens and blood serum using 20  $\mu$ l of protein sample, in liver 30  $\mu$ l and in blood haemoglobin 10  $\mu$ l resulted in best separation of the protein bands. The amount of protein in 20  $\mu$ l of muscle, eye lens, ovary and blood serum and in liver 30  $\mu$ l were calculated to be approximately 200-300  $\mu$ g. Davis (1964) suggested 200  $\mu$ g to be used as sample in each tube.

The techniques to resolve general proteins have been described by Work and Work (1974) and Smith (1976). Three stains i.e., Amido black, Coomassie brilliant blue and Kenacid blue were used in 0.25% concentrations in methanol; double distilled water : acetic acid mixture in the ratio of 5:5:1. Best resolutions were obtained by using kenacid blue stain and 7% acetic acid as destaining solution. Shrinkage

of gels was observed after staining and destaining in methanol; double distilled water : acetic acid mixture. Kenacid blue and Coomassie brilliant blue were found to be more sensitive than the amido black. Some bands which were lightly stained in Kenacid blue and coomassie brilliant blue stains, could not be obtained in amidoblack stain.

Storage of muscle tissue in the freezer was found to result in loss of protein fractions. However, the protein fraction retained in these stored tissues were comparable with corresponding fractions of fresh tissue. The number of bands lost increased with increase in period of storage. It was observed that storage produces denaturation of weak protein bands which appear less intensely stained on the gel.

One of the most effective ways of improving resolution into individual components is variation of the composition of the separating gel. Migration of proteins due to their net charge in the electrical potential applied along the gel will depend primarily on their retention by molecular sieving (Gabriel, 1971). The proteins of high molecular weight will be separated best by using gels of larger pore size, whereas smaller proteins will separate better with gels of smaller pore size.

A gel containing 7-7.5% acrylamide has the most desirable mechanical properties and is commonly used for

protein ranging in molecular weights from 104 to 106 (Gabriel, 1971).

In the present study, of the five different acrylamide concentrations (5.0, 6.3, 7.0, 7.7 and 10.0) tested, best resolutions were obtained in 7.0% acrylamide concentrations in gels. Clear and distinct protein bands and gels were obtained at 7.0% acrylamide concentrations, while other concentrations resulted in poor resolution.

Bouck and Ball (1968) used 5% acrylamide gel and Vonwyll (1980) used 5.5% acrylamide gel, to resolve Ldh. Tsuyuki and Roberts (1965) and Ivanenkov (1980) used 5.5%, 6.0% and 7.0% acrylamide gels to resolve serum proteins for comparing tilapias and their hybrids.

pH, chemical composition, concentration and ionic strength of a buffer play an important role in separation of protein and enzymes. Electric charge of an enzyme is neutral when pH of the buffer is far away from isoelectric point of the enzyme. At this pH, enzymes get precipitated and stabilized. The ionic strength also controls the mobility. The greater the ionic strength, the slower the mobility of charged molecules. However, at reduced migration separation of the enzyme is always sharper.

In the present study, best resolution with maximum number of bands and sufficient interspacing was found at pH

8.9 for muscle, liver, blood serum and ovary proteins, while eye lens and blood serum gave maximum bands at pH 8.5. However, maximum clarity in the gels and bands was found at pH 8.9 for eye lens proteins and blood haemoglobin also.

Studies on separation of enzymes in different continuous and discontinuous buffer systems reveal that Tris-HCl Glycine buffer of pH 8.9 and 8.4, was the best to separate Ldh, Est and Tris-maleic-EDTA at pH 7.4, for Acph. Good resolutions were found for Adh, Mdh and Me enzymes also in the buffer Tris-HCl-glycine and pH 8.9/8.4.

Tris-HCl-Glycine (pH 8.9/8.4) has been used to resolve Ldh (Vonnely, 1979, Bouck and Ball, 1968), Est (Ivanenkov, 1980) and Me (Gould, 1968). Guyomard (1978) used Tris-maleic-EDTA (pH 7.4) for Mdh and Smith *et al.* (1983) for Mdh and Est. Alcohol dehydrogenase has been resolved by using Tris-EDTA-Borate (pH 9.0) and Tris-Citrate LiOH-Boric (pH 7.0) (Guyomard, 1978, Guyomard and Krieg, 1983).

After standardization of the technique, the expressions of the proteins and enzymes in tissues were studied. Proteins (General, lipo and Glyco) and enzymes such as alcohol dehydrogenase, malate dehydrogenase, malic enzyme, lactate dehydrogenase, esterase and acid phosphatase were studied. The obtained tissue expressions for proteins have been discussed in relation to size, sex, maturity stage and species. For

enzymes however, only that tissue in which there was maximum expression of bands has been taken to discuss the similarities and dissimilarities between tested and other fishes. Banding patterns obtained for different isoenzymes have also been discussed in terms of the number of genetic loci present. Tsuyuki et al., (1965) studied the muscle proteins of approximately 50 species of fishes and reported that protein patterns were of species specific nature and independent of age, sex, or maturity stages. Similar patterns have been reported in rock fishes (Tsuyuki et al., 1968), in Tilapia (Hines et al., 1971) and in mullets (Hersberg and Pasteur (1975).

Studies on muscle protein of both sexes of E. surtensis and E. maculatus at different stages of maturation revealed that their patterns were virtually constant in number of bands and species specific. The number of bands remained same in both the species. However the staining intensities of bands decreased in older fishes. Species specific nature of the muscle protein patterns observed in the present study confirms the findings of previous studies on other fishes. The intraspecific variation found in the present study is not in agreement with the report on Gadus morhua (Connel, 1953); Salmon (Nyman, 1967) and brown trout and rainbow trout (Haen and O'Rourke, 1969), where the change in muscle protein fractions with age have been reported.

The only information available for liver protein pattern in fishes is that on Channa stewartii and Danio dangila (Bhattacharya and Alfred, 1982). It has been reported that the liver protein fractions were species specific with the intensities of bands varying as the fish grows.

In the present study on liver protein fractions of E. suratensis and E. maculatus species specificity and constancy between the sexes were found. This agrees with the findings of C. stewartii and D. dangila (Bhattacharya and Alfred, 1982). However, it varied with increase in size of the fish. Appearance and disappearance of protein fractions were attributed to the protein synthesis during development in the course of metabolism.

Species specificity in the eye lens protein have been reported in scombroids (Barret and William, 1967) and in Irish fresh water fishes (Haen and O'Rourke, 1969). Tsuyuki et al. (1968) and Meneses, (1976 a) reported that the eye lens protein patterns were genus specific only in Rock fishes and in Sardinella respectively. Haen and O'Rourke (1969) reported changes due to age in eye lens protein fractions of rainbow trout.

In the present study clear differences were found in the number, position and intensities of eye lens protein fraction

between E. suratensis and E. maculatus. However these patterns were independent of sex, size or maturity stages.

Blood serum protein patterns have been reported to be species specific in salmonids (Tsuyuki and Roberts, 1965) in Tilapia (Avtalion et al., 1975, 1976); in flat fishes and Sardinella (Menezes, 1976a, b; 1979). Sex and age differences in serum proteins have been reported in puffer fishes (Yamashita, 1968), in Sebasticus marmoratus (Yamashita, 1969), in Pseudopleuronectes (Aida et al., 1973). Occurrence of a specific antigen has been reported in maturing females of all salmonid species submitted to immunoelectrophoresis (Utter et al., 1974). Similar observations have been made by Plack and Fraser (1970), in Gadus morhua; Aida et al., (1973) in Plecoglossus altivelis and Kirsipuu (1975) in Esox lucius. They considered these proteins as female specific proteins (Ovovitelline).

The onset of sexual maturity causes both qualitative and quantitative biochemical changes in fish tissues. This is due to the changes in physiology usually characterised by a "reproductive drain", with the diversion of materials for the gonadal development. Naurial and Singh (1985) reported the disappearance of some protein bands during the developing stages of ovary. In the present study, appearance and disappearance of protein bands have been observed during different



stages of development of ovary in both E. suratensis as well as E. maculatus, which can be attributed to protein synthesis during development. Blood serum protein pattern differences associated with maturation has been reported in Petrocyon marinus (Thomas and McCrimmon, 1964); Pseudopleuronectes americanus (Pesch, 1970) and in Cyprinus carpio (Masarekar and Pai, 1979).

In the present study, blood serum protein of E. suratensis showed clear difference between the sexes and during different maturity stages of female fishes. No variations could be observed between different maturity stages of males. Blood serum protein fractions of 1st stage of female fishes were identical in mobility and numbers with those of all stages of male fishes. However, marked variation occurred from the second stage onwards.

The difference in blood serum protein pattern between sexes and appearance and disappearance of protein fractions during different stages of maturation in female was presumed to be caused by a metabolic difference between the processes of vitellogenesis and of spermatogenesis. It is suggested that this difference may be due to the process of yolk accumulation in females.

Species specificity of blood haemoglobins, independent of sex or size have been reported in approximately 50 species

of fishes belonging to Elasmobranchii, Holocephali and Teleostomi (Tsuyuki et al., 1965) in rock fishes (Tsuyuki et al., 1968) in Sebastes (Tsuyuki and Westerheim 1970), and in tilapias (Chen and Tsuyuki, 1970, Hines et al., 1971). Variations in haemoglobin patterns during development throughout life have been reported in salmonids (Cross and O'Rourke, 1978); in clupeoids (Wilkins and Iles, 1956).

In the present study, blood haemoglobin patterns of E. suratensis showed remarkable variation in different size groups studied. It was observed that these patterns were independent of sex, but related to the size of the fish. These findings were in agreement with the observations on other fishes (Chen and Tsuyuki, 1970).

Lipo-protein and glyco-protein of muscle, liver, eye, lens, ovary, blood serum and blood haemoglobin of E. suratensis and muscle, liver, eye lens and ovary in E. maculatus were studied. The number of lipo-protein fractions in E. suratensis and E. maculatus were 6 and 3 in muscle, 4 each in liver, 6 and 5 in eye lens, 5 and 4 in ovary respectively. Similarly, glyco-protein consisted of 6 and 2 fractions in muscle, 4 and 3 in liver, 5 and 7 in eye lens and 4 each in ovary of E. suratensis and E. maculatus respectively. In blood serum and blood haemoglobin of E. suratensis, 5 fractions of glyco and lipo-proteins were observed. It was seen that glyco and lipo-protein fractions

of different tissues of both species had the same mobility as in general protein fractions of corresponding tissues. Identical mobility of these proteins indicated that they have similar net charge.

Muscle myogen polymorphism have been reported in green-stripe rock fish Sebastes elongatus (Tsuyuki et al., 1968); in sable fish Anoplooma fimbria (Tsuyuki et al., 1965 and longnose sucker Catostomus catostomus (Tsuyuki et al., 1967) in Walleye Stizostedion vitreum vitreum (Uthe and Ryder, 1970).

Uthe and Ryder (1970) reported regional variation in muscle protein patterns in walleye. They found variation in observed and expected frequencies of phenotypes from lake to lake and within lakes of Canadian population. However, these were in Hardy Weinberg equilibrium assuming control by two non-dominant alleles.

In the present study, muscle myogen protein showed variation in E. surtensis as well as E. maculatus collected from different geographical areas. Major band No. 10 was common in E. surtensis and in E. maculatus in all areas. Other bands either varied in electrophoretic mobilities or were missing in different areas. Since no uniformity in the band patterns could be obtained, the genetic model could not be evolved and therefore the genetic interpretation for variation, within or between

populations was not possible. In general, though some bands were common with identical mobility in both the species, they were different in their densities and total numbers. Such species-specific character could be attributed to the genetic composition of the individual species. Further it was seen that in the same tissue in each species, mobility of the band remained constant. Similar observations have been made by Barret and Williams (1967) in some scombroid fishes.

Some of the bands occurred with maximum density and showed constancy throughout the size groups or maturation.<sup>1</sup> This indicates that the proteins appeared in the highest concentration at a very early stage of the fish and persisted throughout, a fact attributed to the food availability or habitat, and a key factor in the environment affecting the physiology of fish (Yamashita, 1970). According to Hasche-meyer et al., (1979) and Jurs, (1979) the variation of the bands indicated that protein utilisation was very much correlated to the ambient conditions under which the fish lived. The gradual decrease in the protein suggests that the proteins are utilised as the size of the fish increased to overcome fluctuations in various environmental factors like salinity or temperature affecting fish digestion.

The presence of an isoenzyme in a specialised tissue, or subcellular structure, suggests a specialised role in

metabolism. Keeping this in view, the expression of different isoenzymes, in various tissues of E. suratensis and E. maculatus was determined.

The subunit structure of the isoenzyme, alcohol dehydrogenase has been reported as dimeric in fishes (Wishard et al., 1980a). Liver tissue has been used for genetic analysis in grass carp, big head carp and their F1 hybrids (Magee and Phillip, 1982) and in salmonids (Allendorf 1975; Wishard et al., 1980 a, Guyonard, 1981; Guyonard and Krieg, 1983). Inheritance study showed presence of a single locus for alcohol dehydrogenase in salmonids (Allendorf 1975; Guyonard 1981, Guyonard and Krieg, 1983), in grass carp, big head carp and their F1 hybrids (Magee and Phillip, 1982) in Tilapia and Sarotherodon (McAndrew and Masumdar, 1983).

In the present study the number of bands for the isoenzyme Adh varied among tissues. However, some of the bands were common in different tissues of E. suratensis and E. maculatus. The darkest band was present in the liver tissue of both species, indicating its usefulness for genetic analysis studies. Alcohol dehydrogenase patterns in different tissues of E. suratensis and E. maculatus showed remarkable difference between the two species. No genetic loci were determined in both species.

In fish, malate dehydrogenase is dimeric (McAndrew and Masumdar, 1983; Kirpichnicov, 1981) existing in two forms,

soluble (S) and mitochondrial (M). Each of these forms is coded by an independent gene and in several fish species two or even three genes are involved in the coding of each form.

In fish, tissues such as muscle, liver, eye and heart have been used for expression and genetic analysis of malate dehydrogenase (Wishard et al., 1980 a, b; Guyonard, 1981; Magee and Phillip, 1982; Guyonard and Krieg, 1983; Smith et al., 1983). Allendorf (1975) reported presence of two loci MDH-1 and MDH-2 and Bailey et al., (1970) reported MDH-3 and MDH-4 variable locus in rainbow trout.

Species specificity in malate dehydrogenase patterns have been shown by Basasibwaki (1975) and McAndrew and Masumdar (1983) in cichlid fishes. In E. suratensis identical banding patterns of malate dehydrogenase were seen in muscle, eye and ovary and similarly in brain and intestine, while in other tissues, patterns were entirely different. In E. maculatus more or less similar patterns for malate dehydrogenase were observed in muscle, heart and brain, but in other tissues patterns were entirely different.

The number of MDH-loci present could not be detected due to difference in the number of bands between tissues. However the patterns of malate dehydrogenase observed in both species were easily distinguishable. This is in agreement with the work on other cichlids for their identification (Basasibwaki, 1975; Mc Andrew and Masumdar, 1983).

Malic enzyme also exists in two forms, soluble and mitochondrial, each being encoded by a single locus in many vertebrates. Allendorf *et al.* (1975) and Cross *et al.* (1979) reported tetrameric structure of malic enzyme and existence of five numbered sets of Me bands in heart extracts from brown trout (Salmo trutta) and atlantic salmon (Salmo salar) respectively.

Muscle and liver tissue have been used for genetic analysis of Me in many fishes (Wishard *et al.*, 1980 a, b; Guyonard and Krieg, 1983; Smith *et al.*, 1983).

In the present study malic enzyme patterns of both species E. suratensis and E. maculatus differed between tissues. Both loci as well as the best tissue could not be detected for Me in both species. However, the patterns of malic enzyme observed in different tissues of both species could be used to distinguish the two species.

Lactate dehydrogenase is one of the best studied enzymes in fishes and other vertebrate species. It exists in multiple molecular forms which are presumed to be tetrameric molecules. The patterns of LDH is quite specific for each individual organs and show species differences. Hence the study of LDH isoenzyme patterns is important from the point of view of intermediary metabolism, population genetics, ontogenic change and adaptation to environment (Market and Moller, 1959).

The tissue extracts of E. suratensis and E. maculatus were examined to determine the LDH patterns. Majority of tissues examined in both species, namely muscle, liver, ovary, spleen, kidney, heart, intestine, stomach, testis and blood serum show a single locus. Eye tissues consisted of 5 isoenzymes of LDH, the slowest and fastest moving bands which were darkest, were designated as LDH-1 and LDH-5, which corresponds to A4 and B4 homotetramers respectively. LDH-2, LDH-3 and LDH-4 isoenzymes have intermediate mobility between the most anodic LDH-2 and the most cathodic LDH-1. The brain tissue in both species consisted of 4 isoenzymes of LDH i.e., LDH-1, LDH-2, LDH-3 and LDH-4.

The presence of 5 isoenzymes in E. suratensis and E. maculatus indicated that they possess the two fundamental LDH-loci namely LDH-A and LDH-B. So these fishes exhibit the 5, A and B subunit containing isoenzyme characteristic of higher vertebrates and fishes (Bailey and Wilson 1968). Similar observations have been made on flat fishes, (Markert and Holmes, 1969) sockeye salmon (Hodgins et al., 1969) and cyprinids (Valenta et al., 1977 b).

Tissue specificity has been reported in many fishes such as speckled trout and the lake trout (Goldberg, 1965, 1966), Fundulus heteroclitus (Place and Power, 1979), tench, crucian carp and carp (Valenta et al., 1977 b) and 3 species of trout



(Bouck and Ball, 1968) A single major isoenzyme present in all tissues except eye and brain of E. suratensis and E. maculatus resembles the patterns of LDH in flat fishes (Lush et al., 1969) with a single major isoenzyme in most tissues except central nervous system.

Muscle, liver, eye and heart have been used by various research workers for expression of lactate dehydrogenase and genetic analysis purposes (Wishard et al., 1980 a, b; Guyomard, 1981; Magee and Phillip, 1982; Guyomard and Krieg, 1983; Beck et al., 1983 etc.,). In the present study, all five isoenzymes of lactate dehydrogenase were resolved in eye tissues only, hence eye was used to study polymorphism.

Chen and Tsuyuki (1970) differentiated mouth brooder and substratum spawner Tilapia and Sarotherodon based on their lactate dehydrogenase patterns. Similarly species specific nature of lactate dehydrogenase patterns have also been reported in South American cichlids (Schohl and Hersberg, 1972) 5 lake victorian cichlids (Basasibwaki, 1975); tench, crucian carp, carp (Valenta et al., 1977 b); and Xenopus sp. (Vomwyl, 1979).

In the present study the electrophoretic patterns of LDH in different tissues of E. suratensis and E. maculatus were virtually identical. However, they differed in electrophoretic mobilities. Thus both species can be differentiated

based on the electrophoretic mobility of their isoenzymes. This is in agreement with the work on other fishes.

Studies on the lactate dehydrogenase pattern of eye tissue in E. guratensis showed polymorphism at LDH-1 locus as its allelic frequencies were less than 99% ( $P < 0.99$ ). Faster locus LDH-2 was monomorphic. The polymorphic loci possessed three alleles LDH-1 (100), LDH-1 (95) and LDH-1(93). The phenotypes of the homozygotes. AA possessed five isoenzymes, while the heterozygote AB and AC possesses six isoenzymes each. The sixth isoenzyme nearer to the cathode was presumed to be the co-dominant allele of the Ldh-1 (100), while the other isoenzyme which was nearer to the cathode was presumed to be the heteropolymer of Ldh-1 (100) and Ldh-1 (93). No homozygotes were observed for slow bands of heterozygotes (Plate XXVII, Fig. 41).

Hochachka (1966) found Ldh muscle specific sub-units D and E in salmonids, which he suggested might have evolved from sub-unit A by gene duplication and mutation. Lactate dehydrogenase B and C observed in eye tissue of E. guratensis resemble closely and D and E muscle Ldhs of Salmonids. Thus Ldh B and C can be considered to have evolved from locus A.

Various populations have been described to be polymorphic. Polymorphism of lactate dehydrogenase has been reported in

several populations of fishes such as Fundulus heteroclitus (Whitt, 1970; Place and Power 1979), Negrin (Leptorhombus whiffiagonia) (Dando, 1970) and Cyprinids (Valente *et al.*, 1977 b). However, the populations were in Hardy-Weinberg equilibrium.

Studies on the Ldh phenotypes observed in E. suratensis revealed that there was highly significant difference in observed and expected frequencies of phenotypes ( $P < 0.01$ ), which indicated the deviation from Hardy-Weinberg equilibrium. A deficit in heterozygosity was also observed.

The esterase enzymes are also one of the best studied genetic markers especially for analysing the onset of gene activation during development. Genetic polymorphism of esterase has been studied in a number of species using tissues such as muscle, liver and blood serum. (Kohn *et al.*, 1971; Ridgway, 1970, Metcalf *et al.*, 1972; Krajnovic and Zikic, 1975 and Wishard *et al.*, 1980 a, b). Some of these esterase variants have proved to be useful markers in fish population studies because gene frequencies differed in isolated populations.

In the present study, the maximum number of bands were resolved in liver tissues of both E. suratensis and E. maculatus. Liver tissue was found suitable in both species due to expression of maximum esterase bands.

Tissue and species specificity of esterases have been reported in white crappie (Pomoxis annularis) and their F1 and F2 interspecific hybrids (Metcalf et al., 1972). In the present study also, esterase patterns of muscle, liver, eye, ovary, blood serum, spleen, kidney, heart, brain, intestine, stomach and gills of both E. suratensis and E. maculatus were species and tissue specific.

A phenotype distribution in accordance with the Hardy Weinberg equilibrium has been reported for several fish species (Ridgway et al., 1970; Koehn and Rasmussen, 1967; Krajnovic and Zikic, 1975). However deviation from Hardy Weinberg equilibrium has been found at the esterase locus, in 14 different populations of Metropia straminea (Koehn et al., 1971).

In the present study, three phenotypes were observed in esterases of liver tissues of E. suratensis and there was considerable difference between the expected and observed phenotype distribution values. The differences were highly significant ( $P < 0.01$ ). An increase in heterozygosity was also observed indicating the deviation of population structure from Hardy-Weinberg equilibrium.

Liver tissues of E. maculatus consist of 6 phenotypes. In this species also, considerable variations were observed between observed and expected value of phenotype distribution indicating a deviation from Hardy-Weinberg equilibrium. These

differences were highly significant ( $P < 0.01$ ) and a deficit in heterozygosity was also found at esterase locus in this species.

Acid phosphatase or orthophosphoric monoesters phosphohydrolase is found in nearly all human and animal cells. Phosphatase catalyses the hydrolytic cleavage of phosphoric acid esters. It is found in kidney, liver, spleen, erythrocytes and blood plasma. Minute amounts of the acid phosphatase have also been found to occur in the pancreas, skeletal and heart muscles and in the mucosa of small intestine (Hollander, 1968).<sup>Igarashi &</sup>

Beckman *et al.*, (1968) studied in separation of acid phosphatase in tissue extracts. Four bands namely, A, B, C and D were recognised. A, B and D bands predominated in the liver, intestine, heart and skeletal muscle, B in the skin and D in the pancreas. The C components were present in large amount in placenta.

In the present study acid phosphatase was observed in all tissues of *E. suratensis* as well as *E. maculatus*. Activity of Acph in liver was greater than in other tissues of both species. However, the electrophoretic mobilities and intensities varied among tissues and between species, indicating tissue and species specificity.

Sub unit structure of Acph has been reported to be dimeric in vertebrates (Ward, 1977). This enzyme is considered

as monomorphic by rule, hence isoenzymes are not considerable. A single locus has been reported in trout (Wishard et al., 1980b) and two loci in Cichlasoma cyanostrigatum (Kornfield and Koehn, 1975).

In E. suratensis 4 patterns of Acph were observed in liver tissues, two homozygotes and heterozygotes each. Due to disuniformity in the bands of heterozygotes, variation could not be related to genetic model.

Acid phosphatase patterns in the liver tissues of E. maculatus consisted of three phenotypes, with two homo and one heterozygotes. No significant differences in the observed and expected values of phenotypes was found. This indicated that the phenotypes were distributed in Hardy-Weinberg equilibrium. These differences were not significant ( $P > 0.05$ ). A deficit in heterozygosity was also observed which was also not significant ( $P > 0.05$ ).

Kirpichnikov (1981) suggested that the excess of heterozygosity may be the consequence of negative assortative mating or it may be the result of selection against one of the alleles. The excess heterozygosity observed for Ldh and Est loci in E. suratensis may be attributed to this hypothesis.

A deficit in heterozygote was observed in E. maculatus at Est locus but the phenotypes observed at Ldh locus showed

distribution in Hardy Weinberg equilibrium and non significant deficit was observed.

The deficit of the heterozygotes may be the outcome of the mixing of populations, inbreeding, positive assortative crossing or finally a consequence of the selection operating on the heterozygotes (Kirpinchikov, 1981).

Deficit in heterozygote observed at Est locus in E. maculatus may also be attributed to this hypothesis. It is unlikely that the deficit of heterozygotes observed in phenotypes of esterase in E. maculatus is due to positive assortative mating. This may be explained by the fact that differential selection against the heterozygote would lead to an unstable situation, resulting in fixation of one or the other allele, unless selection is very weak and interpopulation mixing is great.

The common observation in fishes is that the inbreeding causes heterozygote deficiency in biochemical genetic system (DeLigny, 1969).

If the heterozygote deficiency observed at Est locus in E. maculatus is due to inbreeding in local sub populations, similar variation should be demonstrated at other polymorphic loci, such as Acph locus. The present observations do not support this view because the distribution of phenotypes at

AcpH locus in this species was at Hardy-Weinberg equilibrium. The magnitude of heterozygote deficiency observed at the esterase locus is quite large. However, if this was due to inbreeding, deficiency should be detectable at AcpH locus. It is unlikely therefore that inbreeding is responsible for the Est heterozygote deficiency.

The mixing of non-uniform group of fishes (sub-populations and different age groups) differing in their allele frequencies results in the Wahlund effect (Wahlund, 1928, quoted by Kirpichnikov 1981). i.e., an increase in the number of homozygotes and a decrease in the heterozygotes. This Wahlund effect has been discussed above with reference to the very large deficiency of Est heterozygotes in *E. maculatus*. Large samples from different populations are necessary to demonstrate the degree of mixing of the populations.

A deficit of heterozygotes is indeed frequently observed in fish populations, since pure panmictic groups are quite rare. When a population is uniform, and different age groups have different gene frequencies, the number of heterozygotes may be less than expected (Kirpichnikov, 1981).

Total amount of genetic diversity within a population is estimated by average heterozygosity values. This measure can provide an insight into the population history and



structure and the amount of genetic interchange achieved by migration. It is however necessary to analyse several loci, at least 20-25 loci and then determine the average heterozygosity (Utter et al., 1974). Interpretation with statistics is only then possible, to arrive at its implications.

An initiative has been made with the present study in assessing the genetic variation in the population of E. suratensis and E. maculatus from Cochin. However, the present study is confined to only two polymorphic loci each in both species. At least 20-25 loci are necessary to quantify the average heterozygosity and the magnitude of total genetic variation of the species.

## 7. CYTOTAXONOMIC STUDIES

### 7.1. Introduction

Fishes exhibit great morphological variability and one might expect a great diversity in their karyotypes. However, this has not been found to be wholly true. Several orders and families have been found to possess similar karyotypes. For example, 48 acrocentric chromosomes are common in many species, particularly in the comparatively recent order perciformes (Sola *et al.*, 1979) and the heterogeneous nature of the group is revealed amply in the complex classification (Greenwood *et al.*, 1966). This most interesting group, however, has not been well understood cytologically. Of the 32 orders, containing more than 400 families, the chromosome numbers of only 57 families falling under 13 orders are at present known.

Study of fish chromosomes have been few and spotty, most of the early observations being related to spermatogenesis or development. (Turner, 1919; Geiser, 1924 Hann, 1927; Bannington, 1936). A little information is available on chromosome numbers of cichlids also. Post (1965) reported chromosome numbers of ten species, Ohno and Alkins (1966) Nishikawa *et al.*, (1973) and Prasad and Manna (1976) reported on one species each. Badr and El-Dib, (1977a) studied

the chromosomes of three species, Michele and Takahashi, (1977) of 2 species and Thompson, (1979; 1981) of 47 species of cichlids. Karyotype studies of Indian cichlids E. suratensis and E. maculatus have been made by Natarajan and Subramanyan (1974) and Rishi and Singh (1982).

Evidence suggests that the karyotype can play a primary role in speciation (White, 1978) contrary to Mayr's original hypothesis (1963), which stated that only polyploidy and geographical isolation lead to the formation of new species (quoted by Sola et al., 1979).

Keeping in view the above fact the present study on chromosome was undertaken on E. suratensis and E. maculatus inhabiting different environments of peninsular India.

## 7.2. Methods

The squash method and air drying method were used for karyotype study.

### Squash method (Badr and El-Dib, 1977 b)

- a) The animals were measured and weighed.
- b) An intra-muscular injection of 0.1 cc per 10 gms. body weight of a 0.05% colchicine solution was given. Injection was given 50 minutes prior to the time of killing the fish for examination.
- c) Tissues such as testis, spleen, kidney and the gills were dissected out and cut into small pieces about 3 mm<sup>3</sup> in size.

- d) The tissues were kept in double distilled water at pH 7.0 for 15 minutes for swelling of cells.
- e) The tissues were then fixed in 50% acetic acid for 30 minutes at room temperature and later stored in 70% alcohol at 4°C for 1 hour.
- f) Stored tissues were allowed to warm up to room temperature and then transferred to 1N HCl at room temperature for 2 minutes.
- g) Tissue hydrolysis was performed in 1N HCl at 50°C for 15 minutes.
- h) The tissues were washed in distilled water and stained in Schiff's reagent for half to one hour.
- i) Tissues were removed and a slurry of cells was scraped onto a microscope slide. A fresh drop of stain was added and cover slip was applied.
- j) After removing air bubble from beneath coverslip, a piece of filter paper was placed over slide and tissue was squashed using gentle rolling pressure from thumb.
- k) The coverslip was ringed with nail polish to make semi-permanent preparations.
- l) Slides were examined under the microscope.

The problems encountered with this method were:

- Adequate swelling of cells was not obtained in some cases
- Only a few single cells were observed and tissues were clumped.
- Cells were not burst and chromosomes were therefore not spread.
- Metaphase plates were not clear

**Modification of squash method:**

- a) Duration of hypotonic treatment was increased to 20, 30 and 40 minutes. KCl (0.4%) and sodium citrate (1.0%) were also tried for hypotonic treatment.
- b) Tissues were cut into smaller pieces ( $1 \text{ mm}^3$ ) to increase swelling rate.
- c) In cases where chopping proved to be inadequate and clumps of tissues were observed, the material was minced in 45% acetic acid for 30 minutes and cell suspension was used for squash preparation.
- d) Stain aceto-orcein (1%) was used instead of Schiff's reagent.
- e) Methanol: acetic acid mixture (3:1) ratio was used for fixative with variable time for 15 minutes, 30 minutes and 45 minutes.
- f) After hypotonic treatment and fixation, the tissues were put in 50% acetic acid for 30 minutes, later stained and squashed.
- g) Various duration of colchicine treatment from 30 minutes to 17 hours were tried to get clear metaphase plate in a single cell. Gill and scale epithelium tissue were taken from the specimens swimming in the colchicine solution.

**Air Drying Method:**

(Based on Gold, 1974 and Kliegerman and Bloom, 1977)

- a) Fish was allowed to swim in 0.005-0.01% colchicine solution for 6-18 hours. Colchicine (0.01% in 0.85% sterile NaCl) solution was also injected intraperitoneally for larger specimens.

- b) Fishes were killed and testis, spleen and kidney were dissected out. Gills and scale epithelium were removed from the specimens swimming in colchicine solution.
- c) Pieces of tissues were kept in 2 ml of 0.4% of KCl solution for 20, 30, 40 and 50 minutes with frequent changes after every 5 minutes.
- d) Hypotonic solution was pipetted off and tissues were fixed in 3:1 methanol acetic acid solution for 30 minutes with 3 changes each. Fixative was made immediately before use.
- e) Pieces of tissues were removed, blotted lightly on filter paper to remove excess fixative and placed on a cavity slide in 2-3 drops of 50% acetic acid.
- f) Tissue was minced with scalpel or fine dissecting needle for a minute to make a cell suspension and remaining tissue fragments were replaced in fixative.
- g) A drop of cell suspension was drawn using a fine pasteur pipette and expelled on to an acid cleaned slide heated at 40-50°C.
- h) Drop was sucked back in to pipette leaving a ring of cells and it was repeated a few times to leave a number of concentric rings.
- i) Slides were stained in Giemsa in 0.1 M phosphate buffer (pH 6.8) for 10 minutes. Excess stain was washed off in distilled water.
- j) Cover slip was put over the slide to examine the wet mount.

The problems encountered in the method were:

- The single cells were few in number and clumped in peripheri of the ring.
- Cells and chromosomes were not well spread.
- Metaphase plates were not clear

Modifications of Air-drying method:

- a) The tissue was homogenised immediately after removing from animals, instead of chopping (Milligan, 1976)
- b) The slurry of fixed cells was dropped from a height of 2 feet on to hot slides inclined at 60° to facilitate the spreading of the cells and chromosomes.
- c) Distilled water and sodium citrate 11.0% have been tested as hypotonic solution with varied time.
- d) Various duration of colchicine treatment from 30 minutes to 17 hours were tried to get metaphase plate in a single cell.
- e) Aceto-orcein (2.0%) was used for staining.
- f) Slurry of cells were also dropped on chilled slides kept in refrigerator and the air drying was done.

### 7.3. Results and discussion

In the squash and air drying method, the gills were identified as the best tissues, showing faster swelling with more number of single cells than any other tissues studied.

Distilled water was the best for hypotonic treatment with 25 minutes for gill tissues, whereas for spleen, kidney and testis, the best results were observed when treated for 60-80 minutes.

Fixation in 70% alcohol caused over-swelling and distortion of chromosomes, hence a 3:1 mixture of methanol : acetic acid was used.

Hydrolysis with 1N HCl followed by rinsing in distilled water reduced the clarity, hence this step was omitted. Chopping and squashing in glacial acetic acid provided the best spread of cells. Therefore after fixation, the tissues were put in to 50% glacial acetic acid, before staining and the slurry of cells was used for spreading over the slides.

Staining in 2% aceto-orcein for 5 minutes produced better result than staining in Giemsa stain or Schiff's reagent.

In air drying method the homogenisation proved to be better than mincing, hence the homogenisation of tissues followed by centrifugation for air drying method was used, while mincing provided better results in squash technique. Thus the hypotonic treatment of 25 minutes for gills in distilled water, fixation in 3:1 mixture of methanol: acetic acid followed by 50% glacial acetic acid treatment



for bursting the cells and staining in aceto-orcein (2.0%) for 10 minutes were used for all the experiments in squash and air drying methods.

Various treatment in different timing of colchicine could not yield the clean metaphase plate in a single cell to enable the count and study of the morphology of chromosomes in both species, hence the experiment was terminated.

## 8. INDUCED BREEDING AND REPRODUCTIVE POTENTIALS

### 8.1. Introduction

The methods of traditional fish culture practiced in India has been to some extent replaced in recent years by the high yielding aquaculture technologies. The major constraint in fish culture, however appears to be the scarcity of quality fish seed of the cultivated fishes.

The outstanding success of developing hypophysation technique of cultivated Indian major carps (Catla catla, Labeo rohita, L. calbasu, Cirrhinus mrigala, (Chaudhuri and Alikunhi, 1957) and Chinese carp (Ctenopharyngodon idellus) (Cuv & Val) (Hypophthalmichthys molitrix) Cuv & Val (Alikunhi *et al.*, 1963) for production of quality seed has not only opened dependable source of fish seed, but also brought a revolutionary effect in fish culture in India. This method of artificial propagation has been successfully applied in certain catfishes (Ramaswami and Lakshmanan, 1959; Khan, 1972 a,b) and in mullets (Mugil macrolepis, and Mugil cephalus (Alikunhi *et al.*, 1971; Sebastian and Nair, 1978; Chaudhuri *et al.*, 1977). But still there are a large number of species which cannot be bred in captivity.

Seeds of Cyprinus carpio, the common carp are produced mainly by breeding under controlled conditions with or without hypophysation.

Seeds of brackish water fishes like mullets, *Chanos*, *Lates*, *Polynemus* and *E. suratensis* are obtained from the natural sources. Traditional method of fish culture in brackish water involves allowing the seeds of various species to enter an enclosure along with the high-tide, where number of each species and their composition are not known. Commercial seed production of these species could not be done so far by hypophysation. However induced breeding technique of grey mullets *Mugil cephalus* and *M. macrolepis* have been standardised (Chaudhuri *et al.*, 1977, Alikunhi *et al.*, 1971; Sebastian and Nair, 1978). While in the latter, several batches of fry were produced, in the former no fry could be produced on a large scale due to critical, rearing problems.

Attainment of optimum stage of gonadal maturity in brood stock is pre-requisite for successful induced breeding operation. The cultivated major carps mature along with the increasing photo-period and temperature. The maturation process starts in February and completes in April-May, when the day length is beyond 12 hrs. Fish use day length as a reliable clue for the time of their gonadal recrudescence prior to the onset of monsoon rains, which triggers the release of gonadotropin from the pituitary, leading to spawning under natural conditions in river. This phenomenon repeats itself every year in a cyclic order but in pond environment these species do not attain full sexual maturity and thus normally fail to breed in such an environment.

E. suratensis, fairly common in fresh and brackish water, breeds in wild and no information is available on its breeding in captivity except the report of Panikkar (1924) about the fish breeding in an aquarium tank. Present attempt was to induce the fish to breed in plastic pools in the laboratory by hormonal injection and to study the reproductive potential. Panikkar (1924) reported E. suratensis to breed in an aquarium seven times by removing spawns each time. Lamon (1984) (pers. comm. 1984) bred this fish in an aquarium in salinity 10‰, and temperature 26-28°C.

## 8.2. Methods

Experiments were conducted with live specimens kept in two sizes of plastic pools, one with 3' x 2' and the other 12' x 4'. Salinity of water was maintained at 12.0‰, and temperature between 28-30°C through out the experiment. Only healthy specimens collected were transferred to the experimental plastic pools after acclimatization. Two males with a female in small plastic pools and 4 males with two females were kept together in bigger pools. Small aquarium was provided with a bed of sand gravel and a medium sized flower pot, whereas in bigger tank two plastic troughs containing sand gravel were kept at some distance from each other. A brick shelter closed on three sides and the top and open on one side for entrance of fish, was provided in each tank.

The total length and the weight of the ripe females ranged between 170 to 235 mm and 155.5 to 231.0g respectively. For males, the range was between 135 mm to 181 mm and 71.5 to 129 gms. All fishes were conditioned for a day before being subjected to the hormonal treatment.

In the laboratory, the fish were sexed by examining the anal papilla. The occurrence of ripe female was determined by siphoning of intra-ovarian eggs by a plastic cannula. Spawning condition of ripe males was determined by the indication of free flow of milt, when slight pressure was applied on the abdomen. The quantity of the hormone to be injected to the individual fish was determined based on the stage of maturity and body weight of the fish. A total of 2-4 injections were given in the dorsal musculature of the fish at an interval of 24 hrs. Fishes were treated with two hormones separately; Human chorionic gonadotropin (HCG), Pregnant mare gonadotropin (PMG) and two steroids 17- $\alpha$  Methyl testosterone and 17- $\beta$  Oestradiol.

Dry method of stripping (Chaudhuri *et al.*, 1977) was followed in artificially fertilizing the extruded eggs with the milt when the fish failed to spawn. The fertilized eggs were kept in glass bowls provided with swift stream of air bubbles. They were also distributed in glass beakers and small glass troughs containing the water of the salinity of

the experimental pools. All the containers were provided with aeration.

### 8.3. Results and discussion

Out of 6 experimental groups of fishes 4 were treated with Human chorionic gonadotropin (HCG) hormone and 2 with pregnant mare gonadotropin (PMG) during December 1984 and January 1985. Both males as well as females were given hormone injection of HCG (Table 58). No courtship behaviour could be observed even after providing the conditions similar to the natural environment for shelter and no spawning took place naturally. Hence the eggs were stripped off from females and milt was also obtained from males which were kept in glass bowls and gently agitated with the help of a feather. Fertilization did not take place. Few eggs were found to be yellow opaque and darkly pigmented, while the eggs in matured stage were found to be of yellowish brown in colour. In some cases some transparent eggs were also noticed.

Two experiments using different dosage of pregnant mare gonadotropin hormone (PMG) also resulted in atresia in females, so it was not possible to fertilize the eggs with sperms artificially in PMG treated specimens.

During February and March 1985 attempts were made to induce the fishes for spawning by treating with steroids like

TABLE - 58 INJECTION DOSE, SCHEDULE AND REACTION OF E. SURATENSIS TO GONADOTROPIN AND STEROIDS.

Sex	Length mm/ weight g.	Time of the injection and dosage/Kg.Body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - I</u> HUMAN CHORIONIC GONADOTROPIN							
Male	145.0/ 85.5	17.00 19.12.84 1000IU (HCG)	17.00 20.12.84 1000IU (HCG)	17.00 21.12.84 1500IU (HCG)	17.00 22.12.84 1500IU (HCG)	No response in the first and second injection. Good response after 3rd & 4th injection	Spawners were moving actively
Male	150.2/ 90.6	17.10 19.12.84 1000IU (HCG)	17.10 20.12.84 1000IU (HCG)	17.10 21.12.84 1500IU (HCG)	17.10 22.12.84 1500IU (HCG)	No response in I and II injection. Good response after 3rd & 4th injection.	Spawners were moving actively.
Female	217/231	17.20 19.12.84 2000IU (HCG)	17.20 20.12.84 2000IU (HCG)	17.20 21.12.84 4000IU (HCG)	17.20 22.12.84 4000IU (HCG)	Belly became larger after 3rd injection and moving actively but after 4th injection fish became lethargic	No spawning stripped after 4th injection & fertilization attempted artificially.

Sex	Length mm weight g.	Time of the injection and dosage/Kg. Body Weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - II PREGNANT MARE GONADOTROPIN</u>							
Male	173/116.2	17.40 19.12.84 1000IU (PMG)	17.40 20.12.84 1000IU (PMG)	17.40 21.12.84 1500IU (PMG)	17.40 22.12.84 1500IU (PMG)	Ova slightly transparent. No response even after 4th injection.	Fishes became sluggish after 3rd injection
Male	160/108.5	17.50 19.12.84 1000IU (PMG)	17.50 20.12.84 1000IU (PMG)	17.50 21.12.84 1500IU (PMG)	17.50 22.12.84 1500IU (PMG)	No response even after 4th injection	Fishes became sluggish after 3rd injection.
Female	181/139.4	18.00 hrs 19.12.84 2000IU (PMG)	18.00 hrs 20.12.84 2000IU (PMG)	18.00 hrs 21.12.84 4000IU (PMG)	18.00 hrs 22.12.84 4000IU (PMG)	No response even after 3rd & 4th injection	No pairing & no spawning Atresia noticed



Sex	Length mm weight g.	Time of the injection and dosage/Kg.body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - III HUMAN CHORIONIC GONADOTROPIN</u>							
Male	176/115	18.00hrs 2.1.85 1000IU (HCG)	18.00 hrs. 3.1.85 1500IU (HCG)	18.00 hrs 4.1.85 1500IU (HCG)	18.00 hrs 5.1.85 1500IU (HCG)	Fish was moving actively after 2nd injection.	No pairing no spawning
Male	181/129.0	18.20 hrs 2.1.85 1000IU (HCG)	18.20 hrs 3.1.85 1500IU (HCG)	18.20hrs 4.1.85 1500IU (HCG)	18.20 hrs 5.1.85 1500IU (HCG)	Fish was moving actively after 2nd injection.	Fish died after 2 4th injection.
Female	195/163	18.30 Hrs 2.1.85 2000IU (HCG)	18.30 Hrs 3.1.85 3000IU (HCG)	18.30 Hrs 4.1.85 4000IU (HCG)	18.30 Hrs 5.1.85 5000IU (HCG) Followed by 5th & 6th injection on 6.1.86 & 7.1.86.	Belly became large after 2nd injection & moving act- ively but no spawning took place. Ova were slightly transparent.	Eggs were stripped off & attempted to fertilize artificially.

Sex	Length mm/ Weight g	Time of the injection and dosage/mg. Body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - IV</u> PREGNANT MARIANADOTROPIN							
Male	170/112.0	18.50 Hrs. 2.1.85 2000IU (PMG)	18.50 Hrs. 3.1.85 2000IU (PMG)	18.50 Hrs 4.1.85 2000IU (PMG)	18.50 Hrs 5.1.85 2000IU (PMG)	No response even after 4th injection	No pairing & no spawning
Male	175/108.5	18.60 Hrs 2.1.85 2000IU (PMG)	18.60 Hrs 3.1.85 2000IU (PMG)	18.60 Hrs 4.1.85 3000IU (PMG)	18.60 Hrs 5.1.85 3000IU (PMG)	No response after 3rd injection. Fish died after 4th injection.	No pairing & no spawning
Female	206/220	19.05 Hrs 2.1.85 5000IU (PMG)	19.05 Hrs 3.1.85 6000IU (PMG)	19.05 Hrs 4.1.85 8000IU (PMG)	19.05 Hrs 5.1.85 10000IU (PMG)  Followed by 12000 IU on 6.1.85 and 14000 IU on 7.1.85 of (PMG)	No response even after 6th injection.	Fish became sluggish & & died after 6th injection.  Atresia noticed

Sex	Length mm weight g.	Time of the injection and dosage/Kg.body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - V</u> HUMAN CHORIONIC GONADOTROPIN							
Male	168/115	18.30 hrs 18.1.86 1000IU(HCG)	18.30 hrs. 19.1.86 1500IU(HCG)	18.30 hrs 20.1.86 1500IU(HCG)	18.30 hrs 21.1.86 1500IU(HCG)	Fish was moving actively after IIInd injection	No pairing & courtship
Male	173/109	18.45 hrs 18.1.86 1000IU(HCG)	18.45 hrs 19.1.86 1500IU(HCG)	18.45 hrs 20.1.86 1500IU(HCG)	18.45 hrs 21.1.86 1500IU(HCG)	Fish was moving actively after IIInd injection	No pairing & courtship
Female	210/117	19.00 hrs 18.1.86 2000IU(HCG)	19.00 hrs 19.1.86 4000IU(HCG)	19.00 hrs 20.1.86 6000IU(HCG)	19.00 hrs 21.1.86 8000IU(HCG)	Belly enlarged after Ist inje- ction but no spawning. Ova were slightly transparent	No pairing & courtship Fertilizat- ion attempted artificially but there was no further development.
Female	198/160	19.15 hrs 18.1.86 2000IU(HCG)	19.15 hrs 19.1.86 4000IU(HCG)	19.15 hrs 20.1.86 6000IU(HCG)	19.15 hrs 21.1.86 8000IU(HCG) followed by 10000IU(HCG) on 22.1.86 & 12000IU(HCG) on 23.1.86	Belly enlarged after Ist inje- ction, however fish became sluggish after 6th injection and died on 7th day.	No pairing and courtship Atresia noticed.

Sex	Length mm weight g.	Time of the injection and dosage/Kg. Body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - VI</u> HUMAN CHROINIC GONADOTROPIN							
Female	205/178	18.00h 24.1.86 2000IU (HCG)	18.00h 25.1.86 3000IU (HCG)	18.00h 26.1.86 4000IU (HCG)	18.00h 27.1.86 5000IU (HCG)	Ova diameter increased after 3rd injection but no further development with the 4th	Atresia noticed
Female	192/170	18.15h 24.1.86 2000IU (HCG)	18.15h 25.1.86 2000IU (HCG)	18.15h 26.1.86 4000IU (HCG)	18.15h 27.1.86 4000IU (HCG)	Ova diameter increased after 3rd injection but no further development with the 4th	Atresia noticed
Female	176/120	18.30h 24.1.86 3000IU (HCG)	18.30h 25.1.86 6000IU (HCG)	18.30h 26.1.86 8000IU (HCG)	18.30h 27.1.86 12000IU (HCG)	Good response after 1st injection but after 4th injection no further development.	Atresia had set in after 4th injection
Female	181/139	18.45h 24.1.86 4000IU (HCG)	18.45h 25.1.86 6000IU (HCG)	18.45h 26.1.86 8000IU (HCG)	18.45h 27.1.86 10000IU (HCG)	Slight development noticed after 2nd, 3rd & 4th injections. Afterwards no further development even after 6th dose	Atresia noticed after the 6th injection
				Followed by 5th and 6th injection on 28th & 29.1.86 respectively			

Sex	Length mm weight g.	Time of the injection and dosage/Kg. body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - VII MELETHYL TESTOSTERONE</u>							
Male	176/123.5	18.00h 4.2.85 50 mg	18.00h 5.2.85 75 mg	18.00h 6.2.85 75 mg	18.00h 7.2.85 100 mg	No response even after 4th injection	No pairing and spawning
Male	160/89	18.10h 4.2.85 50 mg	18.10h 5.2.85 75 mg	18.10h 6.2.85 100 mg	18.10h 7.2.85 100 mg	No response even after 4th injection	Eggs were stripped & fertilisation attempted.
Female	199/182	18.20h 4.2.85 75 mg	18.20h 5.2.85 100 mg	18.20h 6.2.85 100 mg	18.20h 7.2.85 100 mg	No response even after 4th injection. Ova slightly yellow and transparent	No further development

Sex	Length mm weight g.	Time of the injection and dosage/Kg.body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
M							
EXPERIMENTAL GROUP - VIII METHYL TESTOSTERONE							
Male	156/92	18.40h 4.2.85 100 mg	18.40h 5.2.85 150 mg	18.40h 6.2.85 150 mg	18.40h 7.2.85 150 mg	No response even after 4th injection.	No pairing with female
Male	150/85.9	18.50h 4.2.85 100 mg	18.50h 5.2.85 150 mg	18.50h 6.2.85 150 mg	18.50h 7.2.85 200 mg	No response even after 4th injection	Eggs were stripped & fertilisation attempted no further development could be observed.
Female	170/155.5	19.00h 4.2.85 100 mg	19.00h 5.2.85 150 mg	19.00h 6.2.85 150 mg	19.00h 7.2.85 200 mg	No response even after 4th injection. Ova were slightly yellow and transparent	"

Sex	Length mm weight g.	Time of the injection and dosage/Kg. body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - IX</u> METHYL TESTOSTERONE							
Male	176/115	18.00h 20.2.85 150 mg	18.00h 21.2.85 150 mg	18.00h 22.2.85 200 mg	18.00h 23.2.85 200 mg	No response even after 4th injection	No pairing was observed with females
Male	181/127	18.15h 20.2.85 150 mg	18.15h 21.2.85 150 mg	18.15h 22.2.85 200 mg	18.15h 23.2.85 200 mg	Fish became sluggish and died on 5th day	Eggs were stripped & fertilisation attempted
Female	198/174	18.30h 20.2.85 150 mg	18.30h 21.2.85 150 mg	18.30h 22.2.85 200 mg	18.30h 23.2.85 200 mg	Fish became inactive on 4th day. Ova slight yellowish and transparent.	but no further development was observed

Sex	Length mm weight g.	Time of the injection and dosage/Kg. body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		

EXPERIMENTAL GROUP - X METHYL TESTOSTERONE

Male	156/95	18.40h	18.40h	18.40h	18.40h	Fish became sluggish after 3rd injection and died after 4th injection	No pairing was observed with females
		20.2.85	21.2.85	22.2.85	24.2.85		
		150 mg	200 mg	250 mg	250 mg		
Male	145/80.0	18.50h	18.50h	18.50h	18.50h	Fish became sluggish after 3rd injection & died after 4th injection	
		20.2.85	21.2.85	22.2.85	23.2.85		
		150 mg	200 mg	250mg	250 mg		
Female	187/163.2	19.00h	19.00h	19.00h	19.00h	Fish died after 4th injection	Atresia noticed
		20.2.85	21.2.85	22.2.85	23.2.85		
		150 mg	200 mg	250 mg	250 mg		



Sex	Length mm Weight g.	Time of the injection and dosage/Kg.body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP-XI</u> 17B CESTRADIOL							
Male	170.5/120	18.00h 3.3.85 25 mg	18.00hrs 4.3.85 40 mg	18.00hrs 5.3.85 50mg	18.00hrs 6.3.85 50 mg	No response even after 4th inject- ion.	No pairing with female could be observed.
Male	159/89.0	18.15h 3.3.85 25 mg	18.15h 4.3.85 40 mg	18.15h 5.3.85 50 mg	18.15h 6.3.85 50 mg	No response even after 4th inject- ion	Eggs were stripped off ferti- lization and atten- pted but no further development
Female	235/208	18.30h 3.3.85 25 mg	18.30h 4.3.85 50 mg	18.30 5.3.85 50 mg	18.30h 6.3.85 75 mg	No response even after 4th inje- ction.	

Sex	Length mm weight g.	Time of the injection and dosage/Kg. body weight				Response	Remark
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - XII 17B-CESTRADIOL</u>							
Male	180/125	18.45h 3.3.85 50 mg	18.45h 4.3.85 75 mg	18.45h 5.3.85 75 mg	18.45h 6.3.85 100 mg	No response even after 4th injection	No pairing with female could be observed
Male	163/95.0	19.00h 3.3.85 50 mg	19.00h 4.3.85 75 mg	19.00h 5.3.85 75 mg	19.00h 6.3.85 100 mg	No response even after 4th injection	Eggs were stripped and fertilization attempted.
Female	195/160	19.15h 3.3.85 75 mg	19.15h 4.3.85 75 mg	19.15h 5.3.85 100 mg	19.15h 6.3.85 100 mg	No response even after 4th injection	But no further development could be observed

Sex	Length mm weight g.	Time of the injection and dosage/Kg. body weight				Response	Remark
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - XIII</u> 17 $\beta$ -CESTRADIOL							
Male	158.5/105	18.00h 17.3.85 125 mg	18.00h 18.3.85 140 mg	18.00h 19.3.85 150 mg	18.00h 20.2.85 150 mg	No response even after 4th injection	No pairing with female could be observed.
Male	145/80.5	18.15h 17.3.85 150 mg	18.15h 18.3.85 150 mg	18.15h 19.3.85 175 mg	18.15h 20.3.85 175 mg	No response even after 4th injection	Eggs were stripped and fertilization attempted.
Female	178/150	18.30h 17.3.85 125 mg	18.30h 18.3.85 150 mg	18.30h 19.3.85 150mg	18.30h 20.3.85 175 mg	No response even after 4th injection	Daily but no no further development.

Sex	Length mm weight g.	Time of the injection and dosage/Kg.body weight				Response	Remarks
		Ist	IIInd	IIIrd	IVth		
<u>EXPERIMENTAL GROUP - XIV</u> 17 $\beta$ -OESTRADIOL							
Male	144/75.0	18.45 h 17.3.85 150 mg	18.45h 18.3.85 150 mg	18.45h 19.3.85 175 mg	18.45h 20.3.85 175 mg	No response even after 4th injection	No repairing with female could be observed
Male	135/71.5	19.00h 17.3.85 150 mg	19.00h 18.3.85 150 mg	19.00h 19.3.85 175 mg	19.00h 20.3.85 175 mg	No response even after 4th injection	Eggs were stripped and fertilization attempted
Female	190/180	19.15h 17.3.85 175 mg	19.15h 18.3.85 175 mg	19.15h 19.3.85 200mg	19.15h 20.3.85 200 mg	No response even after 4th injection	No further development was observed.

Methyl testosterone and 17 B estradiol, respectively. Fishes treated with different dosages of Methyl testosterone (50 mg-200 mg/Kg) did not show any response and courtship was not observed, hence the eggs and sperms were stripped off from the specimens and were subjected to artificial fertilization. No fertilization of the eggs could be observed. Specimens treated with higher dosage of methyl testosterone (150 mg-250 mg/kg) resulted in atresia in female and males died after 3rd injection of 250 mg/kg dose, whereas female died after 4th dose of the same quantity.

Specimens treated with 17-B oestradiol did not show any response of courtship, so the eggs and sperms were stripped off for fertilization. No fertilization could be observed in the eggs of specimens treated with this hormone. The details of the experiments are presented in the table 58.

The use of mammalian steroids and gonadotropins for the induced breeding of fish offers a number of advantages over fish pituitary homogenates or extracts. They are readily available, are uniform and consequently can be calculated exactly for potency and dosage, and they avoid the need to sacrifice sexually mature fish for pituitary material.

Human chorionic gonadotropin, hormone has been reported to induce ovulation and spawning in Heteropneustes fossilis

(Sunderraj and Goswami, 1966), in Carassius auratus (Yamazaki, 1965), in Plecoglossus altivelis (Ishida, 1972) in Mugil cephalus (Shehadeh *et al.*, 1973, Kuo and Nash, 1975, James *et al.*, 1983).

Bhowmick (1978) reported that use of 'Synahorin' (Human chorionic gonadotropins 25 IU with 4 mg pituitary/kg of fish weight) was effective in inducing spawning in Labeo rohita, but when 'synahorin' was used alone negative results were obtained. Similar results were obtained by Bhowmick (1979) and Singh *et al.*, (1979) in L. rohita and Cirrhinus idella respectively. In the present study also spawning could not be induced in E. suratensis by treating with different dosages of hormones like HCG and PMG. Babikar and Ibrahim (1979) reported in Tilapia nilotica that HCG (1200-12,000 IU per kg body weight) induced ovulation and increased the degree of hydration of ovary, whereas the Pregnant mare gonadotropins (PMG) was not effective.

Experiments in male Mugil cephalus demonstrated that 17 methyl testosterone was effective in inducing spermatogenesis during and outside the breeding season. (James *et al.*, 1983) In the present study, treatment with 17 methyl testosterone did not show any response towards spawning. No courtship behaviour could be observed in the specimens.

In *Eilapia nilotica*, Oestradiol 17 B was most effective at the dose level of 50-250 mg/kg body weight for ovulation (Babikar and Ibrahim, 1979). Gilbert (1967) suggested that oestradiol - 17B is not directly involved in the processes of ovulation and spawning but modulates the development of the oocytes in preparation of ovulation. In the present study treatment with oestradiol - 17 B could not induce spawning in *E. suratensis*.

Panikkar (1924), Ward et al., 1976 a,b) and Lamon (1984) (Pers. comm.) have reported *E. suratensis* to breed in aquaria without any hormone injection. Thampi (1978) suggested that, since the fishes breed in Kerala during the periods of low salinities and high salinities, this parameter might have some effect on their spawning.

## 1. SUMMARY

1. The work aims to clarify the taxonomic status of Indian Cichlids E. suratensis and E. maculatus through studies of morphometric, meristic and protein pattern variations of fishes from different geographical areas of peninsular India, carried out from Dec. 1981 - Dec. 1985. Attempts have been made to understand the genetic make-up of populations of both species in Cochin backwaters through isoenzyme study and to see the variability in biochemical constituents of fish tissues during the maturation cycle. Study on chromosomes and induced breeding has also been made.

2. A review of previous literature on various studies such as food and feeding, breeding and physiology and taxonomy of Indian cichlids has been presented.

3. For morphometric and meristic studies the specimens of E. suratensis were collected from Cochin, Mangalore, Karwar, Goa, Pondicherry, Madras (Muthukadu and Pulikat lake) and Hyderabad; and E. maculatus from Cochin, Madras (Muthukadu and Pulikat lake) and Hyderabad. The data on morphometric characters were computed and mean, standard deviation, correlation matrix as well as variance - covariance matrix were obtained for each character studied at different localities. The analysis of dispersion was applied to nine characters to see the variations in respect of these



characters in different localities. The data on meristic characters were subjected to 't' test to see their variations in different localities.

4. Morphometric and meristic studies reveal that the populations of E. suratensis from the brackish water of Cochin and the fresh water lake of Hyderabad showed no significant variation in respect of morphometric and meristic characters and hence considered as homogeneous populations. Similar homogeneity was observed in the populations from Karwar and Pondicherry.

5. Brackish water populations of E. suratensis from Mangalore showed homogeneity in morphometric characters along with similar populations of Madras. Karwar and Goa populations between them showed homogeneity in morphometric characters only. Comparison of Mithukadu and Hyderabad populations however indicated homogeneity only in meristic characters, being different in the morphometric characters.

6. Populations of E. maculatus from Cochin, Madras and Hyderabad showed homogeneity in morphometric characters, whereas in meristic characters they were observed to be heterogeneous.

7. The biochemical parameters such as moisture, protein, carbohydrate, lipid and ash were estimated in the blood, muscle, liver and gonads at each maturity stage of E. suratensis and E. maculatus. The data were computed and mean, standard deviation, correlation and partial correlation values for the

biochemical components in different tissues were obtained. A decrease in protein, lipid, carbohydrate and ash content was observed in muscle and liver with the advancement of maturation, which however increased after spawning. Simultaneously an increase in all the above components was observed in the maturing gonads, which however decreased after spawning. The moisture content was observed to increase in muscle and liver and decrease in the gonads with advancement of maturation. After spawning the condition reversed with decrease of moisture content in muscle and liver and increase in the gonads. It is seen that fluctuations in the biochemical composition of these tissues were closely related to the stage of maturation of gonads. On the whole, there was a distinct drain of the body resources to the gonads in both E. suratensis and E. maculatus.

8. The polyacrylamide gel electrophoretic methodology for protein and enzymes separation was standardised to get optimum resolution. Various continuous and discontinuous buffer systems were used for enzyme separation. Buffer Tris-glycine-HCl (pH 8.9/8.4) was observed to be the best for separation of proteins and enzymes such as alcohol dehydrogenase, malate dehydrogenase, malic enzyme, lactate dehydrogenase and esterase, while buffer Tris-maleic-EDTA (pH 7.4) for acid phosphatase only. For best resolution of proteins from most of the tissues, 7% acrylamide concentration in the

gels was found to be the optimum. Protein banding patterns of muscle, liver, eye lens and ovary of E. suratensis and E. maculatus were elucidated.

9. There was no variation in respect of muscle and eye lens protein patterns in different ages, sexes and maturity stages in both E. suratensis and E. maculatus. Protein patterns of liver and haemoglobin of E. suratensis and liver of E. maculatus showed variation with age. However, it did not vary in different sexes. Ovary and blood serum protein patterns of E. suratensis and ovary protein patterns of E. maculatus showed variation with age and maturation. Major protein bands were similar in all the localities. However, variations in minor bands were observed between localities.

10. The expression of various enzymes namely; alcohol dehydrogenase, malate dehydrogenase, malic enzyme, lactate dehydrogenase, esterase and acid phosphatase were obtained in tissues such as muscle, liver, eye, ovary, testis, spleen, kidney, heart, brain, intestine, stomach, gills and blood serum in both species from Cochin.

11. Polymorphism was observed for Ldh and Est in E. suratensis and for Est and Acph in E. maculatus. Significant differences in observed and expected frequencies were found for Ldh and Est in E. suratensis and for Est in E. maculatus. The plausible reasons for these variations have been discussed.

12. Chromosome study was tried using squash and air drying method on both E. suratensis and E. maculatus.

13. Various dosages of hormones such as Human Chorionic Gonadotropin (HCG), Pregnant Mare Gonadotropin (PMG) and steroids such as Methyl Testosterone and 17 B-estradiol were tried to induce E. suratensis to spawn in the laboratory. The dosages used were HCG, 1000-12000 IU; PMG 1000-14000 IU; Methyl Testosterone 50-200 mg and 17 B-estradiol, 150-250 mg per fish depending on its weight. The fishes did not respond to these dosages.

14. The results of the present investigations on morphometric, meristic and biochemical genetic characters of E. suratensis and E. maculatus, throw light on the occurrence of homogeneous and heterogeneous populations.

The existence of homogeneous populations of E. suratensis in widely distant areas and environments like the estuarine areas of Cochin and the land-locked fresh water lake of Hyderabad suggest that by and large the species does not change quickly, morphologically or genetically due to the differing environmental impacts and is able to live, grow and thrive in all these environments. This also implies that the species might prove to be a suitable one to be introduced for cultivation in wider tracts of water bodies of different environmental characters.

15. Heterogeneity is observed in populations like those of Cochin and Mangalore and other sets of geographical areas. The plausible interpretation is that the coastal stocks of Cochin and Mangalore and elsewhere have existed independently for years and developed some variations.

16. Results of the study on biochemical changes during maturation have contributed to the knowledge on the reproductive physiology of the fishes, which will be of help in seed production under controlled conditions and also selection of ideal types of fish for culture.

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